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HYBRIDIZATION AND GERM CELLS OF OENOTHERA MUTANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XCIII

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Since my previous paper[1] went to press, in which were described the conditions of abortive pollen development in *O. lata*, as well as some of the stages in pollen development in the *O. Lamarckiana* hybrid obtained by crossing *O. lata* with *O. Lamarckiana* pollen, further investigations have placed in a new light the situation in regard to the number of chromosomes in the Oenothera mutants and hybrids studied. The further facts obtained have disclosed quite a different condition from the one anticipated, and one which is at present wholly inexplicable, and apparently quite unique so far as chromosomes are concerned, though it is to be expected that fuller knowledge of the facts will bring an explanation of the situation. Since the facts, so far as known at present, appear to be most directly concerned with Oenothera hybrids, a brief account of the forms of hybridization among the Oenothera mutants, as developed by the very extensive hybridizing experiments of DEVRIES,[2] will first be necessary.

FORMS OF OENOTHERA HYBRIDS

In general, DEVRIES' experiments appear to disclose at least three main forms or types of hybridization in the Oenothera mutants: (1) hybrids between a mutant and the parent *O. Lamarckiana;*

[1] GATES, R. R., Pollen development in hybrids of *Oenothera lata* × *O. Lamarckiana*, and its relation to mutation. BOT. GAZETTE **43**:81–115. *pls. 2–4.* 1907.

[2] DEVRIES, HUGO, Die Mutationstheorie, Vol. II. 1903.

(2) hybrids between two mutants; (3) ordinary Mendelian hybrids, which appear in some of the mutant crosses. These can only be considered as general tendencies, however, for the results vary widely in many of the crosses, both in the number and the percentage of the types appearing. DEVRIES has also been able to vary the results experimentally in a number of cases. Crosses in which a mutant is involved he calls mutation hybrids (*Mutationskreuzungen*), and hybrids of exactly this character do not occur, so far as I know, in any other genus of plants or animals.

The conspicuous types of hybridization in Oenothera are as follows: When *O. Lamarckiana* is crossed with a mutant, in the case of several of the forms DEVRIES experimented upon, a hybrid is produced which splits, but differs from a typical Mendelian hybrid in three respects. (1) A heterozygote, showing only the characters of the dominant parent, and containing the recessive characters latent, is not formed in the first hybrid generation, but instead the two parent forms are produced. (2) The proportions of the offspring are not always about 3:1 as in a Mendelian hybrid, but the percentage of plants having the recessive characters (the mutants are recessive to the parent *O. Lamarckiana*) often fluctuates between 0 and 50 per cent., or even with wider range. This distinction is believed to be less important, as fluctuations in the percentage of the forms are known to occur in some Mendelian hybrids. (3) A still more striking difference is that, while in a Mendelian hybrid two-thirds of the dominant plants continue to split in the proportions of 3:1 in successive generations, both the forms produced in a mutation hybrid, or cross of *O. Lamarckiana* with a mutant, breed true and show no splitting. There is thus no external evidence that crossing has ever occurred. This type of hybrid appears when *O. lata*, *O. nanella*, or *O. rubrinervis* is crossed with *O. Lamarckiana*. The following diagram will make clear the distinctions between this and an ordinary Mendelian hybrid. *A* represents the characters of *O. Lamarckiana* in *fig. 1*, and of the dominant parent in *fig. 2*. *B* represents the characters of a mutant in *fig. 1*, and of the recessive parent in *fig. 2*.

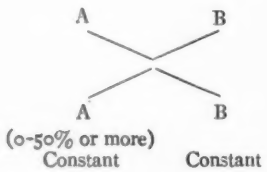


FIG 1.—Mutation hybrid

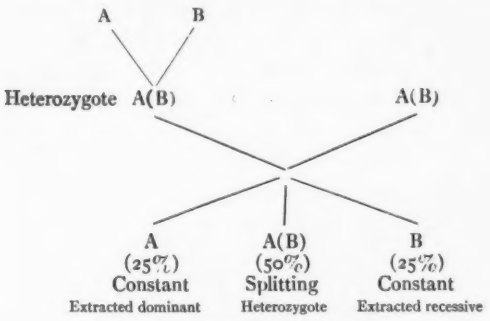


FIG. 2.—Mendelian hybrid

Two significant exceptions to this form of hybrid among the mutants are found in *O. brevistylis* and *O. laevifolia*, which never appeared as mutants in DEVRIES' cultures, although he believes them to have formerly mutated from *O. Lamarckiana*. They form with *O. Lamarckiana* ordinary Mendelian hybrids.

The fact that the percentage of a mutant appearing in a cross can be experimentally varied in different ways is also of importance in this connection. Thus the percentage of the parent forms appearing in a cross varies with the position on the stem of the particular flowers crossed. The plants bloom from below upward on the stem, and DEVRIES observed that after the first few weeks of blooming there is a steady and marked decrease in the size of the flowers blooming, until at the close of the season they are but little larger than those of *O. biennis*. I have observed the same thing in my own cultures from seeds of DEVRIES at Woods Hole, Mass. At the end of September many of the plants were still blooming vigorously at the top, and the flowers were little if any larger than those of *O. biennis*. When pollen from a mutant is used to cross with *O. Lamarckiana*, if it is obtained from the earlier flowers of the season, that is from the larger and more vigorous flowers which are lower on the stem, the heredity-coefficient,[3] or percentage of the mutant appearing in a cross, will be larger than if later and smaller flowers are used. Similarly the heredity-coefficient (*Erbzahl*) of flowers on the side branches of *O. lata* when crossed with *O. Lamarckiana* is said to be less than that

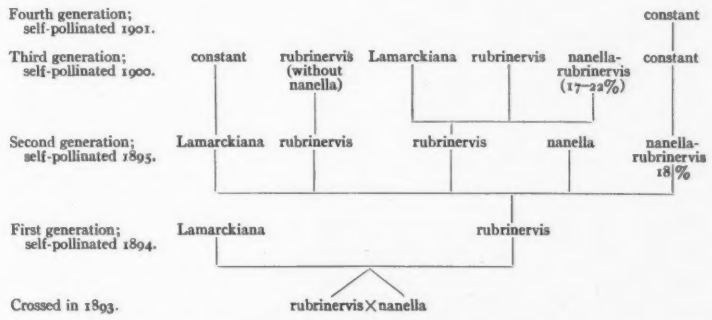
[3] I would suggest this term as an English equivalent for the German *Erbzahl* and shall use it in this sense in the rest of the paper.

of flowers on the main axis (DEVRIES *l. c.* **2**:416). The heredity-coefficient of a mutant, when the large earlier flowers are used, appears to be a fairly constant quantity and to be usually or at least frequently near the Mendelian ratio, though it may often fluctuate in individual crosses of flowers from 0 to about 50 per cent. Thus the heredity-coefficient of *O. lata* is often 21–24 per cent., and that of *O. nanella* is practically the same. This percentage is said to remain about the same from generation to generation and to be independent of the pollen parent; but in crosses of single flowers the percentage may vary widely. Thus 39 crosses of *O. Lamarckiana* flowers were made with *O. nanella* pollen, and the seeds from the different flowers sown separately. The proportions of *O. nanella* obtained, that is the heredity-coefficient, varied from 1 to 48 per cent., the average for the total number of 3768 seedlings being about 17 per cent. (DEVRIES *l. c.* **2**:408).

The proportions of the parents appearing in a cross also fluctuate from other causes. In crossing *O. Lamarckiana* with *O. nanella* pollen, in one experiment when 90–100 seedlings were produced from one ovary the proportion of *O. nanella* was 44 per cent., but when the number of seedlings produced from an ovary was only 20–40, the proportion of *O. nanella* was reduced to 10 per cent. The quantity of pollen used in a cross also influences the offspring, and the proportions may be varied by experimental methods. Thus in one experiment (DEVRIES *l. c.* **2**:417), all of the 4–8 lobes of the stigma of *O. Lamarckiana* flowers were removed except one lobe or a small portion of one. They were then rather sparingly pollinated with *O. nanella* pollen (one flower being used to pollinate several), and of 1593 seedlings produced from 8 *O. Lamarckiana* plants whose flowers were thus treated, all were *O. Lamarckiana*. In another experiment all of the stigma was removed but one lobe, and of 1687 seedlings obtained only about 2 per cent. were *O. nanella*. Other experimental means of varying the results of a cross need not be mentioned here. But that such results can be obtained is of the greatest interest and suggests a line of inquiry as to the nature of the factors determining what forms will appear in a cross. This may also have a bearing on the determination of the chromosome number, though just what the connection may be cannot be guessed at present.

When two mutants are crossed the results show a much greater variation in numbers and forms. In some cases four types are produced in the F_1, though one or more of these types may be absent in any cross. *O. lata*×*O. nanella* may be taken as an example. In one experiment[4] the F_1 showed: *O. lata* 30 per cent., *O. Lamarckiana* 18 per cent., *O. nanella* 47 per cent., *O. nanella-lata* 5(?) per cent. The constant intermediate hybrid type *O. nanella-lata* is usually absent from this cross, and the percentage of the other types may vary widely. The *O. Lamarckiana* and *O. nanella* hybrids from this cross breed true, as does also *O. nanella-lata* when present. Since *O. lata* does not mature its pollen, its constancy cannot be tested by self-pollination.

One other instance of a cross between two mutants (*O. rubrinervis*×*O. nanella*) will be given, to show the complexity and variety of the results.



In this case (DEVRIES *l. c.* **2**:451) *O. Lamarckiana* and *O. rubrinervis* were produced in the first hybrid generation, the latter on self-pollination producing (1) *O. Lamarckiana*, which was constant; (2) *O. rubrinervis*, part of which were pure and part split into *O. Lamarckiana*, *O. rubrinervis*, and *O. nanella-rubrinervis* as in the previous generation; (3) *O. nanella*, whose constancy was not tested; (4) *O. nanella-rubrinervis*, which bred constant through three generations. The form *O. nanella-rubrinervis* is an intermediate type between *O. rubrinervis* and *O. nanella;* it has never appeared as a

[4] DEVRIES, HUGO, Ueber erbungleiche Kreuzungen. Ber. Deutsch. Bot. Gesells. **18**:435–443. 1900.

mutation or in the first hybrid generation, but apparently arises only from self-fertilized hybrids of the second or later hybrid generations.

In these crosses it should be said that, while constancy of the forms appearing in the F_1 is the rule, exceptionally in one of the types which ordinarily breeds true an individual may appear whose progeny repeat the two types of the grandparents, thus showing a tendency to continue splitting (DEVRIES *l. c.* **2**:423).

SHULL'S[5] recent work on hybridization in the elementary species of Bursa (Capsella) shows that these produce ordinary Mendelian hybrids in no way comparable to Oenothera hybrids. In fact I think it is safe to say that no genus of plants or animals is known in which the conditions of hybridization are strictly comparable to those found in Oenothera. This peculiar type of hybridization is not confined to *O. Lamarckiana* and its mutants, but ordinarily appears when a mutant is crossed with another member of the genus, as *O. muricata*, *O. biennis*, *O. hirtella*, *O. suaveolens*, *O. hirsutissima*. In such cases the first hybrid generation usually contains both the parent forms in widely varying proportions, and these in the F_2 are found to breed true. Thus the same type of hybrid is produced as is usual when a mutant is crossed with *O. Lamarckiana*.

The phenomena of hybridization in Hieracium are more nearly like those of Oenothera than any other genus, so far as I know. MENDEL'S[6] original work, continued recently by OSTENFELD,[7] has given us a view of the hybridization conditions in this genus. MENDEL showed in a number of Hieracium crosses that the F_1 is heterogeneous, including some intermediate types and some very near to or indistinguishable from the parents. In the case of *H. praealtum* × *H. flagellare*, the F_2 obtained by self-pollinating the hybrids of the F_1 was shown to be the same as the F_1, all the forms of the latter

[5] SHULL, G. H., Elementary species and hybrids in Bursa. Science **25**:590–591. 1907.

[6] MENDEL, G., Ueber einige aus künstlicher Befruchtung gewachsene Hieracium-Bastarde. Verh. Naturf.-Vereins. Brünn. **8**:26–31 (Abhandlungen). 1869.

[7] OSTENFELD, C. H., Castration and hybridization in the genus Hieracium. Rep. 3d Internat. Conf. on Genetics. Jour. Roy. Hort. Soc. London **24**:285–288. 1900.
———, Experimental and cytological studies in the Hieracia. I. Castration and hybridization experiments with some species of Hieracia. Bot. Tidssk. **27**:225–248. *pl. 1.* 1906.

breeding true. OSTENFELD[8] first showed the presence of apogamy in the genus by castration experiments. MURBECK[9] has since found that the egg in these cases develops the embryo parthenogenetically. The absence of fertilization in some cases is thus a factor at least in the peculiar form of hybridization. OSTENFELD[7] has recently extended these studies to a considerable number of species. Among other species he has shown by castration experiments, confirmed by the cytological investigations of ROSENBERG,[10] that *H. excellens* is partly parthenogenetic and partly develops normally fertilized eggs; its pollen is also sterile. ROSENBERG found in this form the interesting condition which he calls apospory, where a cell of the nucellus enlarges and obliterates the normally formed tetrad of megaspores. This cell completes the embryo sac morphology and the egg develops without fertilization. More rarely apogamous embryo sacs are formed, as in Taraxacum. Usually, however, normal tetrad formation, with reduction of chromosomes, takes place, followed by fertilization. Part of the plants produced in a cross are then pure *H. excellens* produced from apogamous or aposporous embryo sacs and part are real hybrids.

The similarity of this form of hybrid to the *O. lata*×*O. Lamarckiana* cross suggests the presence of apogamy in *O. lata* also, though it is highly improbable that this can explain all the phenomena of hybridization concerned. In the latter cross only the two parent types are produced and the *O. Lamarckiana* breeds true, while in the Hieracium cross several types are produced intermediate between the parents, in addition to the pure *H. excellens*, but experiments of OSTENFELD have not yet been carried far enough to determine the character of later generations, and the number of plants he has

[8] OSTENFELD, C. H., Zur Kenntnis der Apogamie in der Gattung Hieracium. Ber. Deutsch. Bot. Gesells. **22**:376–381. 1904.

———, Weitere Beiträge zur Kenntnis der Fruchtentwickelung bei der Gattung Hieracium. *Idem* 537–541.

[9] MURBECK, S., Parthenogenese bei den Gattungen Taraxacum und Hieracium. Bot. Notiser **1904**:285–296.

[10] ROSENBERG, O., Ueber die Embryobildung in der Gattung Hieracium. Ber. Deustch. Bot. Gesells. **24**:157–161. *pl. 11*. 1906.

[10a]———, Experimental and cytological studies in the Hieracia. II. Cytological studies on the apogamy in Hieracium. Bot. Tidssk. **28**:143–170. *pls. 1–2*. 1907.

obtained from seeds has been in some cases too small for entirely satisfactory results.

The Oenothera and Hieracium hybrids mentioned may be said to resemble each other in that in both the F_1 is heterogeneous, though the types of which the F_1 is composed differ in the two crosses, agreeing only in that one of the types in each is the pure mother parent. The abortive character of the pollen in *O. lata* may also be said to favor rather than otherwise the probability of apogamy in this species, as it is well known that apogamous species frequently do not mature their pollen. This suggestion of apogamy in *O. lata* will be examined in another light in connection with the discussion of the number of chromosomes.

MILLARDET'S[11] "false hybrids" in Fragaria and Rubus, in which only one of the parent types (usually the mother) is produced in the hybrid, and this breeds true, may possibly be regarded as extreme cases of the same type of hybrid as some of the Oenothera crosses.

HURST,[12] among a large number of crosses of orchids which showed many types of hybridization, found eleven crosses between different genera in which only one of the parent types was reproduced, this being in every case the mother (p. 104). Large numbers of the hybrids were grown in some cases. He concluded that in at least eight of these "false hybrids" self-pollination was impossible, and that the embryos probably developed apogamously, perhaps on account of the stimulus from the presence of foreign pollen tubes in the ovary, but without fertilization. Oenothera itself may produce hybrids of this type. Thus DEVRIES found that *O. Lamarckiana* ♀ × *O. biennis* ♂ gives only *O. biennis* in the F_1 and F_2 (*l. c.* **2**:31). This cross made in the New York Botanical Gardens, however, gave in addition to several *O. Lamarckiana* plants, which MACDOUGAL[13] thinks probably came from foreign *Lamarckiana* pollen, four types, none of which were the same as either parent. Thus entirely different results may be obtained under different conditions.

[11] MILLARDET, M. A., Note sur l'hybridation sans croissement ou fausse hybridation. Mem. Soc. Sci. Phys. et Nat. Bordeaux IV. **4**:347–372. 1894.

[12] HURST, C. C., Notes on some experiments in hybridization and cross-breeding. Jour. Roy. Hort. Soc. London **24**:90–126. *figs. 9–41*. 1900.

[13] MACDOUGAL, D. T., Mutants and hybrids of the Oenotheras. Publ. 24, Carnegie Institution, Washington, pp. 56. *pls. 22*. 1905.

The possibilities of apogamy in other mutants are apparently not so good, and such a condition seems rather unlikely, especially in *O. nanella*, where some of the hybridization results practically exclude the possibility of apogamy in these cases. Thus, *O. nanella* ♀ × *O. biennis* ♂ gave (DEVRIES *l. c.* **2**:476) in two crosses 554 plants, none of which were *O. nanella;* while the reciprocal cross gave 96–100 per cent. *O. nanella* among 356 plants. Hence in both cases the pollen parent was practically the only form appearing in the F_1, so that apogamous embryos, at least of any kind known at present, could not have been formed.

THE NUMBERS OF CHROMOSOMES

In my earlier paper[1] it was shown that the sporophyte number of chromosomes in *O. lata* is fourteen, while in the *O. Lamarckiana* plants appearing in the first hybrid generation from a cross with *O. lata*[14] the number is at least twenty. It was assumed therefore that the pure *O. Lamarckiana* parent had at least twenty chromosomes as the sporophyte number. Examination has shown, however, that in the pure *O. Lamarckiana* the sporophyte number is fourteen. This creates a remarkable and unexpected situation, which may be illustrated thus:

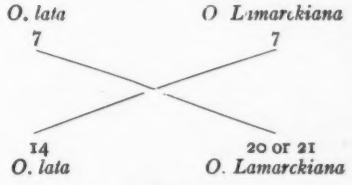


FIG. 3

Two plants, whose gametophyte number of chromosomes is seven, when crossed give rise in the first hybrid generation to the two parent forms, one of which has fourteen chromosomes as the sporophyte number, while the other has twenty or twenty-one.

In *O. lata* the count of chromosomes was made in the pollen mother cells and found to be fourteen. It has since been made in various somatic tissues of the flower, and is found to be constantly

[14] For the sake of brevity I shall refer to the *O. Lamarckiana* plants appearing from flowers of *O. lata* pollinated by *O. Lamarckiana* as "the *Lamarckiana* hybrid."

fourteen so far as observed. There has been no indication whatever that the number is ever higher. In the *Lamarckiana* hybrid it has been found from a number of counts in the floral tissues that the number of chromosomes in the somatic tissues is twenty or twenty-one, and I have found no reason to think it varies. In the pollen mother cells the number of chromosomes in the metaphase and anaphase of the heterotypic mitosis is usually twenty or twenty-one. Occasionally the number in early anaphase appears to be less, but this may be due to a tardy separation of some of the paired chromosomes in the equatorial plate; further study is required to determine this point. A large number of telophases of this mitosis were counted which showed ten chromosomes each. In one case there appeared to be eleven chromosomes in a telophase, though the greater majority at least show ten in each daughter nucleus. Occasionally a chromosome lags behind in the equator of the spindle until late anaphase, and this may make the number twenty-one. Such a case is figured in my former paper (*pl. 4, fig. 39*). This is not always the case, however, as shown by *fig. 38*, in which there are ten chromosomes in each daughter nucleus, but none left behind in the equator of the spindle. It is possible that a single unpaired chromosome might be present in metaphase and be lost in the cytoplasm as the paired chromosomes separated and went to the poles. It is certain that in many pollen mother cells ten chromosomes go into each daughter nucleus in the heterotypic mitosis. Whether the number in the somatic tissues is twenty or twenty-one has not been determined with certainty, as it is a matter of very considerable difficulty. The further discussion will reveal the reason for suggesting that the sporophyte number of chromosomes in the *Lamarckiana* hybrid is twenty-one rather than twenty.

O. Lamarckiana, the parent, has fourteen chromosomes as the sporophyte number, so far as the plants have been examined. This count was made constantly in the floral tissues. The homotypic mitosis in the pollen mother cell shows seven chromosomes constantly so far as observed, a large number of counts being made in various stages of this mitosis. It is doubtless true that chromosome counts as ordinarily made come from a very few individuals. In the case of the *Lamarckiana* hybrid I have examined material from two individuals and from different flowers in each; these plants were grown in

different seasons. In every count the number of chromosomes in the somatic tissues has been twenty or twenty-one, though I have been as yet unable to determine with certainty between these two numbers. Any cytologist is acquainted with the fact that one region of a chromosome group is likely to be obscure enough, owing to close aggregation of the chromosomes, to prevent absolute certainty in making a count of the exact number, unless the number of chromosomes happens to be a small one. The evidence already mentioned shows beyond a doubt that, in the large majority of cases at least, ten chromosomes enter each daughter nucleus in the heterotypic mitosis, though it may be that there are occasionally eleven chromosomes in one of the daughter nuclei.

Several plants of *O. lata* and the pure *O. Lamarckiana* have been examined, all having fourteen chromosomes, which makes it probable that fourteen is the number found in all the pure individuals of these species, and twenty or twenty-one the number found in all the individuals of the *Lamarckiana* hybrid. But it will be necessary to determine the count in several more plants of each of these races before this point can be settled with certainty.

It will be of interest to know whether the number of chromosomes in the *Lamarckiana* hybrid remains constant in later generations, and also what happens when the *Lamarckiana* hybrid having twenty or twenty-one chromosomes is crossed with *O. lata* or with pure *O. Lamarckiana* having fourteen chromosomes. I am now growing plants from crosses made in last season's cultures, which it is hoped will determine these interesting points.

THE HETEROCHROMOSOMES

In my former paper[1] (p. 91), large rings of chromatin were described as sometimes occurring in the pollen mother cells of *O. lata*, cut off from the spirem after synapsis. In the heterotypic mitosis in *O. lata* and the *Lamarckiana* hybrid small bodies are found, ring-shaped (or hollow) but of the same size as the chromosomes, from which they are otherwise indistinguishable. These may be on the spindle or in the cytoplasm, as described in that paper. It was believed that the smaller ring-shaped chromatic bodies were condensed from the larger rings found in the spirem stage. It is possible, how-

ever, that the latter are merely irregularities occurring during pollen degeneration in *O. lata*, for it is well known that a variety of irregularities, in addition to the ones described, may occur in synapsis and the reduction mitosis in sterile hybrids. This leaves unexplained the origin of these bodies called heterochromosomes. On the other hand, the evidence from staining properties and from other considerations, as given in the paper referred to, is against their origin from nucleoli. The same bodies have since been found in the reduction mitoses in *O. Lamarckiana*, where they persist, in some cases at least, through both mitoses to the formation of pollen tetrads. For the present, therefore, the origin and possible significance of these bodies may be considered to be a matter of doubt, in regard to which no conclusions can be drawn until further evidence is obtained.

POSSIBLE EXPLANATION OF THE CHROMOSOME NUMBERS

A number of possible explanations of the origin of the chromosome number in the *Lamarckiana* hybrid will at once occur to botanists. It must be said, however, that all of them appear unlikely, and no explanation can be given on the basis of our present knowledge in regard to chromosome numbers. It is to be expected that further investigation will disclose the sources of the extra chromosomes in the *Lamarckiana* hybrid, but for the present some possibilities may be suggested.

It would seem more probable, because more in accord with present views of chromosomes, that the sporophyte number of chromosomes in the *Lamarckiana* hybrid arises at the time of fertilization by the union of nuclei the sum of whose chromosomes is twenty-one, than that it arose through the subsequent divisions of certain chromosomes. The experimental results of NĚMEC[15] and others, in which somatic nuclei are made to fuse and subsequently divide, showing mitotic figures with chromosomes whose number is the sum of the number of chromosomes in the nuclei that entered the original fusion, indicate the persistency of individual chromosomes in such cases. Such results increase the probability that the number of chromosomes found in the somatic tissues of an individual arose with the genesis

[15] NĚMEC, B., Ueber die Bedeutung der Chromosomenzahl. Bull. Internat. Akad. Sci. Bohême **10**:1–4. 1906.

of the individual and has persisted in all subsequent cell generations. Of course there is the possibility that a higher number of chromosomes might originate through the longitudinal division or the fragmentation of certain chromosomes; but no constant size-differences have been found among the chromosomes of any of the forms or between the chromosomes of the different races examined.

There are a number of ways in which it would be possible for fusion nuclei having twenty-one chromosomes to be formed during the period of fertilization, since the gametophyte number of chromosomes is seven.

(1) It is possible that *O. Lamarckiana* might produce two kinds of pollen, having respectively seven and fourteen chromosomes. My examination of the conditions of pollen development in *O. Lamarckiana* has thus far revealed no evidence whatever in favor of such an hypothesis. Apparently all the pollen mother cells undergo the reduction divisions, in which the number of chromosomes is reduced from fourteen to seven in the usual way.

(2) It is possible that *O. lata* might produce two kinds of eggs, having respectively seven and fourteen chromosomes. If both these kinds of eggs were fertilized with *O. Lamarckiana* pollen and produced embryos, we should have plants resulting with fourteen and twenty-one chromosomes. The difficulty here, however, is that on such an hypothesis the union of seven *lata* chromosomes with seven *Lamarckiana* chromosomes would produce a *lata* plant; while the union of fourteen *lata* chromosomes with only seven *Lamarckiana* chromosomes would produce a *Lamarckiana* plant, a situation which is highly improbable, to say the least.

(3) Another possibility is that all the eggs of *O. lata* have the unreduced number of chromosomes, and that part of them develop without fertilization (parthenogenetically), producing *O. lata* plants with fourteen chromosomes; while others are fertilized with *O. Lamarckiana* pollen, and produce *Lamarckiana* plants having twenty-one chromosomes (*fig.* 3). This assumption is perhaps as reasonable as any, but no case is known of an unreduced egg being fertilized.

(4) Another possible source of twenty-one chromosomes is by the union of both male cells with the egg in fertilization. This, though very unlikely, is at least a theoretical possibility.

(5) It is conceivable that the triple fusion nucleus in double fertilization, instead of the egg, might form the embryo. The only known case of the endosperm developing anything resembling an embryo is Balanophora. TREUB[16] studied *B. elongata* and the same conditions were afterward found in *B. globosa* by LOTSY.[17] Fertilization does not take place in these forms, and the egg and synergids soon degenerate, while the upper endosperm nucleus enlarges and divides, forming a mass of endosperm tissue. In the midst of the endosperm a group of smaller cells is cut off, forming a "pseudembryo," which was followed to the five or ten cell-stage. Tetrad formation apparently does not take place, so that there is probably no reduction of chromosomes, the "pseudembryo" having the $2x$ number.

All of these possibilities, therefore, are without parallel in our present knowledge, but it seems most probable that the source of the twenty-one chromosomes is to be sought in some such situation during fertilization, or the genesis of the individual having this number of chromosomes. It is hoped that further investigation of megaspore and embryo sac development and fertilization in *O. lata* will lead to the explanation of this remarkable situation, though of course the real explanation may be quite different from any of the possibilities here suggested.

On any of these assumptions the number of chromosomes is twenty-one. If it is twenty, however, another explanation must be sought. The correctness of the latter number is supported by the fact that I have counted many telophases of the heterotypic mitosis, in which the number of chromosomes was ten in each daughter nucleus. Also if twenty-one chromosomes originated from the fusion of three nuclei, two of which represented one parent and one the other parent, it might be expected that in synapsis and the reduction mitosis a segregation of the chromosomes would indicate this origin. One might perhaps expect a pairing of seven chromosomes (maternal) with seven others (paternal), leaving the other seven unpaired to pass

[16] TREUB, M., L'organe femelle et l'apogamie du *Balanophora elongata* Bl. Ann. Jard. Bot. Buitenzorg **15**:1–22. *pls. 1–8.* 1898.

[17] LOTSY, J. P., *Balanophora globosa* Jungh. Eine wenigstens örtlich-verwittwete Pflanze. Ann. Jard. Bot. Buitenzorg II. **1**:174–186. *pls. 26–29.* 1899.

to either pole or remain out in the cytoplasm. ROSENBERG[18] found such a situation in the Drosera hybrid, where the ten chromosomes from one parent paired with ten of the twenty from the other parent, while the remaining ten chromosomes of the latter remained unpaired and were irregularly distributed or lost in the cytoplasm. But nothing of this kind has thus far been observed in the reduction divisions of the *Lamarckiana* hybrid, and instead ten chromosomes are apparently regularly distributed to each daughter nucleus in the heterotypic mitosis. It is pretty evident that the exact number of chromosomes cannot be satisfactorily settled until their origin is ascertained.

Not the least remarkable part of this situation is found in the fact that here we have two plants, the pure *O. Lamarckiana* and the *Lamarckiana* hybrid, which cannot be distinguished by their external characters, and yet differ widely in the number of their chromosomes. This difference in number assumes a different aspect, however, if we find, as seems most probable, that we have here merely an extra set of chromosomes from one parent. Such a situation need not necessarily interfere with the present views of persistency of chromosome numbers or even of the ordinary constancy in number for a species. A knowledge of the fate of these chromosomes in subsequent generations and crosses will probably aid largely in determining the general bearing of these facts on present views of the individuality and constancy in number of chromosomes.

It is suggestive that in the *Lamarckiana* hybrid, as ROSENBERG[19a] has found in the apogamous species of Hieracium, the number of chromosomes is much higher than in other species of the genus examined. In the apogamous species in various genera investigated in recent years, where the chromosome numbers are known, the number of chromosomes in apogamous species is nearly or exactly double the sporophyte number in closely related, normally fertilized members of the same genus. A list of these cases need not be given. There may be a suggestion here as to how variation in the chromosome numbers of a genus originates. The same author[19] finds that

[18] ROSENBERG, O., Ueber die Tetradentheilung eines Drosera-Bastardes. Ber. Deutsch. Bot. Gesells. **22**:47–53. *pl. 4.* 1904.

[19] ROSENBERG, O., Cytological investigations in plant hybrids. Rep. 3d Internat. Conf. on Genetics. Jour. Roy. Hort. Soc. London **24**:289–291. 1906.

in *H. excellens* the egg cells have different numbers of chromosomes, owing to irregularities in the reduction divisions. When this form is crossed with another species, the hybrids of the F_1 have different numbers of chromosomes. Whether this is the cause of the different types in the F_1 is not ascertained.

It seems probable, or at least possible, that there is a connection between the type of hybridization in Oenothera and the chromosome numbers, though just what that connection may be is at present quite unknown. Apparently whatever determines the twenty or twenty-one chromosomes determines also that the parent shall have the *O. Lamarckiana* characters. There is thus a possibility that this may be found to have a bearing upon the cause of dominance and recessivity in Mendelian hybrids, the characters of the individual being determined in this case by the presence or absence of the extra chromosomes. CANNON'S[20] suggestion that the Mendelian segregation of characters results from the segregation of maternal and paternal chromosomes in the reduction mitoses is as yet a hypothesis without proof, though ROSENBERG'S[21] recent paper, in which he states that in the pollen development of the Drosera hybrid occasionally two pollen grains of the tetrad have the morphology of one parent and two that of the other, shows that in some cases, at least, segregation of characters takes place at this point in the life history.

Practically the same theory of the segregation of the characters and purity of the germ cells in plant hybrids on the basis of the chromosomes (idants) was put forward by WEISMANN[22] in 1892, in connection with his theory of the germ-plasm. At this time the Mendelian hybrids had not been rediscovered, and complete segregation of the characters of the parental types was regarded as a rare occurrence in hybrids. It may be worth while to quote WEISMANN'S statement (p. 299):

> The germ-mother cells of the hybrid contain a group of idants (chromosomes) derived from the paternal, and another from the maternal ancestral species. If therefore the "reducing-division" halves the germ-plasm of these mother-cells in

[20] CANNON, W. A., A cytological basis for the Mendelian laws. Bull. Torr. Bot. Club **29**:657–661. *fig. 1.* 1902.

[21] ROSENBERG, O., Erblichkeitsgesetze und Chromosomen. Botaniska Studier, Upsala, pp. 237–243. *figs. 5.* 1906.

[22] WEISMANN, AUGUST, The germ-plasm. English translation. New York. 1898.

such a manner that idants of the mother alone reach one ripe germ-cell, and those of the father alone are contained in another, it is possible that two such germ-cells may unite when fertilization takes place between these hybrids. In such a case a plant completely resembling one of the ancestral forms would arise, for it would have been produced from a germ-plasm which contains idants of this species only.

He concludes, however, (on a theoretical basis only) that this rarely happens.

To obtain the Mendelian ratios in hybrids on the basis of the purity of the germ-cells, it is necessary to assume that the mechanism of the reduction mitoses regularly segregates the maternal and paternal chromosomes, and that their distribution to the daughter cells in such cases is not merely a matter of chance, or that a part merely of the chromosomes from one parent can determine the dominance of that form. ROSENBERG[23] has elaborated such a scheme of chromosome segregation in connection with his study of *Drosera rotundifolia* × *D. longifolia*. He found that in the metaphase of the heterotypic mitosis in the pollen mother cell the chromosomes of the two parents, which can be distinguished by their size, are not all oriented, with for example the paternal chromosomes toward one pole of the spindle in the equatorial plate, but some from each parent will enter both daughter nuclei, their orientation on the spindle being thus determined by chance. The Drosera hybrid does not show complete dominance, but is intermediate, some of the characters being nearer to either parent. The character of the hybrid in later generations has not yet been determined.

DEVRIES'[24] conception of the segregation in Mendelian hybrids is that where the parents differ in one unit character this character will be represented in the hybrid by a single *Anlage* from each parent. If these *Anlagen* are assumed to be each represented by a single chromosome, the corresponding members of the other chromosome pairs being qualitatively alike, this would give, as ROSENBERG[23] points out, a chromosome basis for Mendelian segregation of characters. In the formation of the megaspores and microspores, half would contain the chromosome representing the peculiar paternal character, and

[23] ROSENBERG, O., Ueber Reduktionstheilung in Drosera. Meddel. Bot. Inst. Stockholm, pp. 13. *figs.* 20. 1904.

[24] DEVRIES, HUGO, Befruchtung und Bastardierung. Leipzig. pp. 62. 1903.

half the chromosome representing the maternal. The germ-cells would thus all be pure in regard to this character, and in self-fertilization of the hybrid, according to the laws of chance the usual percentages of a Mendelian hybrid would arise.

OTHER CYTOLOGICAL OBSERVATIONS

Several interesting cytological observations, a detailed description of which will be presented at a later time, may be briefly stated here. In the heterotypic mitosis in the pollen mother cells of the *Lamarckiana* hybrid, the usual split in the chromosomes, in the anaphases, which is generally described as a premature split for the homotypic mitosis and is believed by many cytologists to be the reappearance of a split which occurred at the time of synapsis, occurs as described in my former paper. In the early telophases, before there is any indication of a nuclear membrane around the daughter nuclei or of the beginning of a passage into the semi-resting condition, which occurs later, chromosomes having the appearance of distinct tetrads are frequently found. The indications are that this tetrad appearance, which is often very clear and characteristic, may be due to the two halves of a daughter chromosome (which were formed by the split in the anaphases of the heterotypic) remaining side by side and each becoming clubbed at the ends, the portion between these ends in each remaining only as a very delicate connecting thread. This gives the appearance of four spherical bodies of approximately equal size, connected in pairs in one direction by two parallel delicate threads. In other cases the tetrads have quite a different shape, having rather the appearance of a hollow ring drawn out sharply at the four corners. In still other cases, in this telophase, chromosomes are seen having the shape of a pair of spheres close together, or a dumb-bell, and often chromosomes are found at this stage showing no indication of even a double nature, but appearing univalent. This variation in form does not obscure the distinctness of the chromosomes, which are usually clearly distinguishable from each other at this time, and easily counted. All these different forms may be found together in the same nucleus, though so far as observed one form usually predominates in a given telophase. Figures showing these appearances will be published later.

In the homotypic mitosis, as observed in *O. Lamarckiana*, the

chromosomes usually become two-lobed in the anaphases. Just how this shape arises from the pear-shaped chromosomes of the early anaphase has not been determined. In the telophase of this mitosis the chromosomes are nearly always two-lobed or dumb-bell-shaped and can be readily counted (seven in *O. Lamarckiana*) in this condition. They soon become more or less irregular in outline, however, as the nuclei pass into the resting condition, but this characteristic shape usually persists for a short time at least after the formation of the nuclear membrane.

Several interesting features have appeared in connection with the examination of nuclear divisions, in various somatic tissues, a work which is not yet completed. All the tissues examined are flower tissues about the time when the reduction divisions are taking place in the pollen mother cells or later. The conditions so far as observed are identical in corresponding tissues of the three forms examined (*O. lata*, *O. Lamarckiana*, and the *Lamarckiana* hybrid), except for the difference in the number of chromosomes. In the tissue of the anther wall and in the rapidly dividing tissues of the young petals, in both of which a great many chromosome counts were made, the chromosomes are crescents, rods, or loops in metaphase and the early anaphases, the apices of the loops converging in telophase to the poles of the spindle. In the telophase these chromosomes also become two-lobed or dumb-bell-shaped. This shape is very characteristic and apparently constant, or at least occurs very generally at this stage. Observations which are as yet incomplete indicate that in the early telophase these chromosomes begin to appear two-lobed. This shape is very clear a little later, about the time the membrane of the daughter nucleus is formed. The chromosomes can be distinctly counted at this time, and the number is apparently constant in each form. It seems probable that this clubbing of the ends of the chromosomes to form dumb-bells or two-lobed chromosomes in the telophase of the mitoses in these tissues is the same thing that happens in the telophase of the heterotypic mitosis, giving rise to the tetrad appearance, and in the telophase of the homotypic, giving rise to the two-lobed or dumb-bell-shaped chromosomes. Whether this lobing of the chromosomes giving a bivalent appearance is in all these cases a preparation for the split in the next mitosis cannot at present be deter-

mined. The paucity of investigations on the somatic mitoses in plants leaves open the possibility that some of the phenomena of the reduction mitosis to which a special significance has been attached may find their counterpart in ordinary somatic mitoses. It is believed that further study will settle the interpretation of these appearances.

Measurements have shown that the size of the cells, nuclei, and chromosomes in the anther wall is on the average approximately the same as in the petals, though there is considerable variation in the size of the cells of the latter. It is interesting to note that while the chromosomes in the somatic tissues are loops in the metaphase and early anaphases, in the pollen mother cells the chromosomes are usually spherical or pear-shaped in these stages of both the reduction mitoses. This difference is very striking. In the telophase of the homotypic, however, the characteristic shape is apparently the same as in the corresponding stage of somatic mitoses, that is, two-lobed. Measurements indicate that the somatic chromosomes are at this stage considerably smaller than the homotypic chromosomes, though there appears to be much variation in the size of the former.

SUMMARY

1. In my previous paper the sporophyte number of chromosomes in *O. lata* was found to be fourteen, and in the *Lamarckiana* hybrid obtained by crossing *O. lata* with *O. Lamarckiana* pollen, at least twenty. The number of chromosomes is now found to be twenty or twenty-one constantly in all the plants of the *Lamarckiana* hybrid examined, while the number in the pure *O. Lamarckiana*,[25] as well as in *O. lata*, is fourteen, the reduced number being seven. These results are obtained from a large number of counts in various stages of the reduction mitoses and also in the somatic divisions (floral tissues). Thus the pure *O. Lamarckiana* and the *Lamarckiana* hybrid, which are identical in external appearance, differ widely in the number of their chromosomes.

2. The origin of the bodies called heterochromosomes, which are present in the reduction divisions in all the forms studied, is a matter of

[25] J. M. GEERTS, in a short paper just received (Ueber die Zahl der Chromosomen von *Oenothera Lamarckiana*. Ber. Deutsch. Bot. Gesells. **25**:191–5. *pl. 6.* 1907), also finds that the sporophyte number of chromosomes in the pure *O. Lamarckiana* is 14 and the gametophyte number 7.

doubt, and until their mode of origin is ascertained no significance can be attached to them.

3. In the telophase of the heterotypic mitosis in the pollen mother cells of the *Lamarckiana* hybrid, the chromosomes frequently have distinctly the form of tetrads. In the homotypic mitosis of *O. Lamarckiana*, as well as in the somatic mitoses (in various floral tissues) in all forms studied, the chromosomes are two-lobed in the telophase. The tetrad appearance is probably due to the same lobing of the bivalent chromosomes that takes place in the telophase of the homotypic and somatic mitoses.

This investigation has been carried on under the supervision of Professor John M. Coulter and Dr. Charles J. Chamberlain.

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MORPHOLOGICAL AND ANATOMICAL STUDIES OF THE VEGETATIVE ORGANS OF RHEXIA

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(WITH PLATES I AND II)

An anatomical classification of the Melastomaceae has been proposed by VAN TIEGHEM,[1] who divides the family into two tribes: Melastomeae and Memecyleae. In the former the secondary hadrome shows the normal structure, while in the latter the secondary hadrome incloses groups of leptome in as many concentric bands as the stem shows annual rings. The Melastomeae comprise four subtribes: Dermomyelodesmeae, with supernumerary mestome strands in the cortex and pith; Dermodesmeae, with supernumerary mestome strands in the cortex alone; Myelodesmeae, with supernumerary mestome strands in the pith alone; and Adesmeae, without supernumerary mestome strands. The Memecyleae comprise two subtribes: Pternandreae, without sclereids in the leaves; and Mouririeae, with sclereids in the leaves. The genus Rhexia belongs to the Dermomyelodesmeae.

Among other anatomical works may be mentioned those by PFLAUM,[2] PALÉZIEUX,[3] and VÖCHTING,[4] and also a very detailed diagnosis of the family in SOLEREDER'S *Systematische Anatomie der Dicotyledonen* (p. 405).

It would thus appear as if the family had already been treated to such an extent that little if anything of real importance could be added, a fact fully appreciated by the author. However, the study of internal structure is not always accompanied by a corresponding treatment of the external, and so far the Melastomaceae have not

[1] Classification anatomique des Melastomacées. Bull. Soc. Bot. France **38**: 114. 1891.

[2] Anatom.-systemat. Untersuch. d. Blattes der Melastomaceen. Diss. München, 1897.

[3] Anatom.-systemat. Untersuch. d. Blattes der Melastomaceen. Bull. Herb. Boiss. **7**: appendix v. 1899.

[4] Der Bau und die Entwickelung des Stammes der Melastomaceen. Hanstein's Bot. Abhdl. **3**:1. 1878.

yet been thoroughly investigated. The monograph contributed by COGNIAUX[5] is purely systematic and adds nothing to the natural history of these interesting plants. Copious as is the literature, very little attention has been paid to the following questions: how the plants grow, how they develop from seeds to mature plants, and how well the structural characteristics are preserved under various conditions.

Although the family is most widely distributed in America, very few species occur north of the tropics, and Rhexia is the only genus that occurs in the United States and Canada, with about ten species. Two of these, *R. virginica* and *R. mariana*, occur in the District of Columbia, the former extending as far north as Ontario, the latter to New Jersey. They both inhabit sandy swamps, associated with *Lilium superbum*, certain Cyperaceae (Scleria, Fimbristylis, Scirpus, and Carex), Polygalaceae, Violaceae, etc.

Having had the opportunity of studying these two species in the field at various seasons, I have found several points in their external structure which have not hitherto been described, and which may serve as a small contribution to the knowledge of the life-history of these interesting plants. To this is added a brief anatomical description of the vegetative organs, and especially of the roots, since these have not been studied heretofore.

Rhexia virginica L.

While studying the vegetative propagation exhibited by representatives of the local flora, I never was able to find any specimen of this species with a rhizome as described in the various manuals. According to BRITTON, GRAY, and SMALL, this species should possess "horizontal, slender, and tuberiferous rootstocks." That this statement is very incorrect may be seen from the following notes, in which I shall endeavor to show that the underground organs described as tuberiferous rootstocks are simply tuberous roots; and that the development of root-shoots is of great importance to the plant, being its only means of vegetative propagation.

Root-shoots of adult specimens, like those of *figs. 3* and *4*, do not give any clear idea of the remarkable way in which this herbaceous

[5] DeCandolle's Monogr. phanérog. **7**:385. 1891.

plant persists. In *fig. 3* a root-shoot is represented which at first glance may suggest a "slender tuberiferous rootstock" with a basal stem-portion, subterranean and covered with scale-like leaves. In *fig. 4*, however, a larger tuberous body is seen, from which several stems have developed, whose irregular position does not indicate that they have arisen from the axils of opposite leaves. As a matter of fact, there are neither leaves nor leaf scars to be observed upon this tuberous organ. The "rhizome" represented in *fig. 4* is the one most frequently found in flowering or fruiting specimens. A closer examination shows that the tuberous organs are not tubers, but tuberous roots. Moreover, in *fig. 3* the small tuberous body is borne upon a slender branch, which is seen to be a secondary root and not a stolon, since it bears no leaves. If these organs were stems, they would have been provided with opposite leaves like those of the aerial shoots, and the shoots would necessarily have occupied a much more definite position than those shown in *fig. 4*. Moreover, the internal structure of these organs proved to be that of a true root. This is the only type of "rhizome" that I have found in the very many flowering and fruiting specimens examined.

The development of root-shoots is a phenomenon very frequently observed in our herbaceous and woody plants, but thus far we know a very limited number of plants that persist only as root-shoots. It was very desirable, therefore, to study the younger stages and especially the seedlings of Rhexia. On account of their diminutive size they are difficult to find in nature, since they are always more or less hidden by the rank vegetation that covers the swamps. However, I succeeded in detecting some small patches of young plants among which there were many seedlings. One of these is shown in *fig. 1*, which represents the complete root system, the very small epigeic cotyledons (*Cot*), and the basal internodes of the aerial shoot. At this stage the primary root (*R*) is the main root of the young shoot; it bears several lateral and very thin branches, two of which are opposite and developed beneath the cotyledons. These two roots commence to swell, and during the summer they show a local thickening in the shape of a fusiform tuber (*r*). A few secondary roots sometimes develop from the first internode a short distance above the cotyledons. At the end of the first season the shoot dies down to the ground, and

the primary root withers with the exception of the two lateral swollen branches, which winter over as two separate roots.

In the following spring a young shoot develops, with scale-like, opposite leaves at the basal nodes, and with several pairs of secondary roots from the nodes, while a dark-colored tuberous body occurs at the base of the shoot. One or more of the secondary roots soon commence to increase in thickness, forming fusiform tubers like the one described above on the root of the seedling. *Fig. 2* shows a root-shoot; the short, dark tuber at the base of the shoot represents the lateral tuber of the seedling (*r* in *fig. 1*), and the shoot is developed not from the base of the tuber but from its upper face. If the shoot had continued the axis of the swollen root, there would have been a persistent primary root, but such a case was not observed; the shoot invariably occupied the position shown on the root marked *r* in *fig. 2*, where a bud is seen near the base on the upper face of the tuber. This bud is to develop a new and independent plant during the next year.

The specimen thus described (*fig. 2*) represents the root-shoot of the seedling and lives only one year, the persisting part belonging to a secondary root. Older specimens, like those in *figs. 3* and *4*, show the same course of development, but the shoots are more vigorous and the tuberous roots much larger. The secondary roots develop in pairs from the nodes, between the opposite scale-like leaves. These roots are quite long, branch freely, and are able to produce shoots as described above. Although the underground portion of the stem bears several leaves, none of these were found to subtend buds, and the shoot dies down completely at the end of the season. The tuberous roots, on the other hand, may live for three years, if not longer; the large specimen represented in *fig. 4* was fully three years old. When these root-tubers persist for more than two years they develop several shoots (*fig. 4*), and several strong, amply branching, lateral roots.

An over-wintering stage is shown in *fig. 5*. The tuber occupies here a horizontal position, and the shoots for the next year are already visible as buds on small stems with scale-like leaves and quite distinct internodes; the place of development of these minute shoots is along the upper face of the root. It is only when the tuber occupies a more or less vertical position that the shoots show a tendency to push out from near the apex, as may be seen in *fig. 3*.

Rhexia virginica, therefore, exhibits a very singular type of vegetative propagation, that is, one restricted entirely to the development of root-shoots.

THE INTERNAL STRUCTURE OF THE VEGETATIVE ORGANS

The roots

The secondary and lateral roots and the slender portions (apical and basal) of the tuberous roots have the same structure, modified only by age. In the tubers, on the other hand, there is a very different structure, since they remain active for a longer time and are the seat of a continuous development of aerial shoots, vegetative or floral. In the capillary lateral roots the primitive root structure remains unchanged, since they persist only one season. In the slender secondary and stronger lateral roots, which develop from the tuberous portion, the structure becomes changed at an early stage on account of the development of cambial strata from the conjunctive tissue on the inner face of the leptome strands, resulting in an increase in thickness of the stele and a gradual obliteration of the primitive structure. These roots may persist and remain active, therefore, for a longer time than do the capillary roots. However, the increase in thickness of the stele does not always cause a rupture of the surrounding tissues, accompanied by secondary formation (cork and secondary cortex); the endodermis, cortex, exodermis, and epidermis often remain entire for some time. As soon as the development of cork commences (in Rhexia from the pericambium), the peripheral tissue necessarily becomes broken, though without being thrown off.

A much greater increase in thickness is seen, of course, in the tuberous roots. In them, even during the very first period of their growth, the activity of the pericambium is so rapid that the peripheral tissues from endodermis to epidermis are thrown off completely, and are replaced by strata of cork and secondary cortex originating from the pericambium. Hence the structure of the tuberous roots shows a marked deviation from that of the other roots. In *fig. 10* there are shown the several layers of cork (*P*), the broad parenchyma of secondary cortex (*C*), and a continuous circle of cambium strata surrounding a broad cylinder of thin-walled parenchyma in which the long but very narrow rays of mestome radiate from the primitive root-stele.

The number of mestome strands varies in accordance with the thickness of the tuber; the largest number observed was ten, representing the growth of one season.

The minor structure of these various tissues seems to be very uniform. The absence of ducts, stereids, and sclereids makes the structure quite simple. The epidermis is sparingly hairy or perfectly smooth. The exodermis is thin-walled and consists of one layer of cells pentagonal in cross-section, whose walls are not contractile. The cortex is thin-walled throughout and frequently shows radial collapsing; some of the cells contain druids of calcium-oxalate, but no deposits of starch. The endodermis is thin-walled also and shows the Casparyan spots very plainly; it surrounds a continuous pericambium of a single layer. A few strata of more or less thick-walled conjunctive tissue occur in the center of the stele and between the leptome and hadrome. In the tuberous roots the cork is well represented (*figs. 11* and *12*), and the secondary cortex is compact, though exceedingly thin-walled (*C* in *fig. 10*). The secondary leptome (*L* in *fig. 10*) shows a very few cells, and the hadrome (*H* in *fig. 10*) consists of several wide vessels separated by thin-walled parenchymatic tissue and by very broad rays of radially stretched parenchyma between the mestome bundles.

The section shown in *fig. 10* is from the tuberous root of the young shoot shown in *fig. 2*, upon which a small bud is visible. The root is in its second year and shows the enormous increase in proportion to the very narrow primitive stele with the short rays of hadrome (*PH* and *H*). The other section (*fig. 13*) is from an older and much thicker tuberous root, in which the number of vessels is larger and the leptome much better represented (*L*).

Although parenchymatic tissues attain a high development in these tuberous roots, they were never observed to contain any deposits of starch. It is to be noted also that these tissues are peripheral, surrounding the primitive stele, and that the central portion of the stele has no pith, but only a few strata of conjunctive tissue.

The stem

All the internodes are four-winged, while the body of the stem is cylindric. The cuticle is thin, but wrinkled; the cells of the epider-

mis are small, and moderately thickened in the wings. Hairs of two kinds are frequent: short and clavate ones (*fig. 9*), and some very long ones, with slender pluricellular stalk and a small globular head, the apical cells being extended into papillae (*fig. 6*); they both represent glandular hairs. The cortex is thin-walled and contains chlorophyll, besides druids of calcium oxalate; no palisades were observed. A thin-walled endodermis surrounds the stele, which is composed of bicollateral mestome strands, with the medullary rays narrow and thick-walled. The inner leptome represents much larger groups than the outer, and an isolated leptomatic strand (*fig. 14*) occupies the center of the pith. This central leptome may be followed through the entire length of the stem, but in the basal internodes it is accompanied by a few vessels which it partly surrounds; thus the structure becomes approximately hadrocentric. The pith is thin-walled and contains neither starch nor druids.

As a member of the Dermomyelodesmeae, Rhexia possesses also some mestome strands in the cortex, and these occur in this species only in the wings, three in each wing and very small, but containing a few elements of leptome and hadrome.

With the exception of an occasional thickening of the cortex in the wings, like a very feeble collenchyma, there is no mechanical tissue; in the basal internodes the epidermis and cortex are thrown off, while the endodermis remains intact and about four layers of pericyclic cork are developed. It is in these internodes that a central mestome strand occurs, with both leptome and hadrome.

The shoot is terminated by a flower whose peduncle, although a direct continuation of the stem, shows a somewhat modified structure. It is cylindric, but wingless, and the cortex contains no chlorophyll. The mestome strands are bicollateral, but the inner leptome forms an almost continuous band close to the hadrome, while in the stem internodes the inner leptome occurs as several distinctly separate strands. No central leptomatic strand was observed in the pith.

The leaf

The structure is bifacial. The cuticle is wrinkled on the upper face, but does not show any prominent striation. Viewed *en face* the lateral cell walls of the epidermis are undulate on the dorsal

surface but straight on the ventral; the outer cell wall is moderately thickened, and the lumen of the cells is much wider on the upper than on the lower face. Hairs like those described above are frequent, but the clavate ones occur only on the dorsal face, where the stomata also occur. These are level with the epidermis and are surrounded mostly by four cells of the same structure as the other epidermal cells, that is, the lateral walls are prominently undulate. There is a typical palisade tissue of one layer, and beneath this a pneumatic tissue of about four strata with very wide intercellular spaces. Viewed *en face* the cells of the pneumatic tissue are star-shaped, interspersed with roundish cells containing druids of calcium-oxalate. A very different structure is to be observed in the median portion of the blade, where the very broad midrib is located. The cuticle is here wrinkled on both faces, and the lumen of the epidermal cells is narrow. A large colorless parenchyma forms a broad keel underneath the midrib, and is thin-walled except where it borders the epidermis. No true collenchyma, however, is developed in any part of the blade, and no stereome. The midrib has no parenchyma sheath and contains one broad, arch-shaped, bicollateral mestome strand and a small supernumerary (*fig. 15*). This small mestome strand shows the leptome above the hadrome, and is separated from the midrib by a few strands of colorless tissue.

The very prominent lateral ribs show the same structure as the median, being bicollateral and covered by a colorless tissue on both dorsal and ventral face.

Rhexia mariana L.

All the specimens examined proved to be root-shoots. Some of them were quite young and vegetative, but I was unable to detect seedlings.[6] This species differs markedly from *R. virginica* in possessing no tuberous roots. The roots that produce the shoots are horizontal, very long and slender, and several shoots, both vegetative and floral, may be observed upon the same root; the lateral branches are thinner, lighter brown in color, and branch freely. The shoots in

[6] The seedling is described and figured by LUBBOCK in his work on seedlings (1:545), but no mention is made of the further growth or as to the occurrence of root-shoots.

this species are able to persist more than one season, since the buds borne on the basal nodes remain active and produce new shoots in the next year. But, as stated above, young as well as old shoots may be observed among each other on the same root, and consequently on its upper face. Two to four slender secondary roots develop from the basal internodes of the older shoots. No specimen was found in which there was any indication of the primary root, and it would be very interesting to know whether the seedling of this species behaves in the same way as that of *R. virginica*.

THE INTERNAL STRUCTURE OF THE VEGETATIVE ORGANS

The roots

It is only in the very thin lateral roots that the primitive structure may be observed. The epidermis shows but little tendency to produce hairs; the exodermis consists of a single layer of thin-walled, pentagonal cells that are larger than those of the adjoining cortex. The cortex is of five layers of thin-walled cells, with wide, rhombic, intercellular spaces; druids but no deposits of starch were observed. A thin-walled endodermis and a continuous pericambium surround five groups of leptome, alternating with five short rays of hadrome; while the center of the stele is occupied by a narrow cylinder of moderately thickened conjunctive tissue.

In the thicker and stronger roots, lateral or secondary, the cortex frequently collapses radially, and there is increase in thickness; however they do not increase as much as do the roots of the foregoing species, and never become tuberous. Cork is developed when the secondary leptome and hadrome begin to appear, arising from the pericambium.

As stated above, the roots of this species are very long and grow horizontally and in several cases an excentric growth was noted. In old roots the epidermis and exodermis are partly thrown off, and the cortex is collapsed almost throughout. The stele, however, is protected by four or more strata of cork, within which there are stereids scattered in small groups outside the leptome; the secondary rays of hadrome are relatively short and separated from each other by mostly three rows of thick-walled parenchyma, which also occurs in the central part of the stele. No starch was observed.

The stem

The basal internodes are cylindric; those above are obtusely quadrangular, with four very narrow wings; hairs like those described under the foregoing species cover the stem. The cuticle is very prominently wrinkled, and the epidermis is thin-walled. The cortex is broken down in wide lacunes in the basal internode, but solid in the upper internodes. No collenchyma or stereome was found in the stem. There is only one small collateral mestome strand in each of the four wings, and sometimes one or two between them. The stele represents two arches within the slightly convex faces of the stem, and consists of bicollateral mestome strands with much leptome on the inner face of the hadrome. The pith is thin-walled and does not contain any isolated central strand of leptome; neither druids nor starch were observed. Pericyclic cork is developed, but only in the basal internodes.

The leaf

The cuticular striations over the ventral epidermis (*figs. 8* and *16*), which radiate toward the center of the outer cell wall, are characteristic of the leaf structure. The lumen of the epidermis is considerably wider on the ventral surface than on the dorsal, and the stomata (*fig. 7*) are not confined to the latter. They show the same structure as those of the other species, and some were noticed on the ventral face of the leaf-blade. Hairs of both kinds described above are scattered over both faces, and also along the margins. The chlorenchyma shows the same structure as observed in *R. virginica*, but the pneumatic tissue is less open. The midrib lacks the supernumerary ventral mestome strand, but otherwise the structure of the veins is identical with that of the former species.

Summary

Considered from an anatomical point of view, these two species may be distinguished from each other by the following contrasting characters:

R. virginica.—Roots tuberous where the shoots develop; no stereids outside the leptome of the root; stem broadly four-winged, with three mestome strands in each wing; one central mestome strand in the pith; no cuticular striations above ventral epidermis of leaf;

stomata only on dorsal face; midrib with a supernumerary mestome strand; clavate glandular hairs on dorsal face of leaf.

R. mariana.—Roots slender throughout; stereids outside the leptome of the root; stem narrowly four-winged, with one mestome strand in each wing; no central mestome strand in the pith; cuticular striations very distinct above ventral epidermis of leaf; stomata on both faces of leaf; midrib without a supernumerary mestome strand; clavate glandular hairs on both faces of leaf.

Common to both species are the bicollateral mestome bundles of stem and leaf; the absence of specialized subsidiary cells around the stomata; the absence of mechanical tissues (stereome and collenchyma); the occurrence of two types of glandular hairs; the bifacial structure of the leaves; and the ability of the roots to produce shoots.

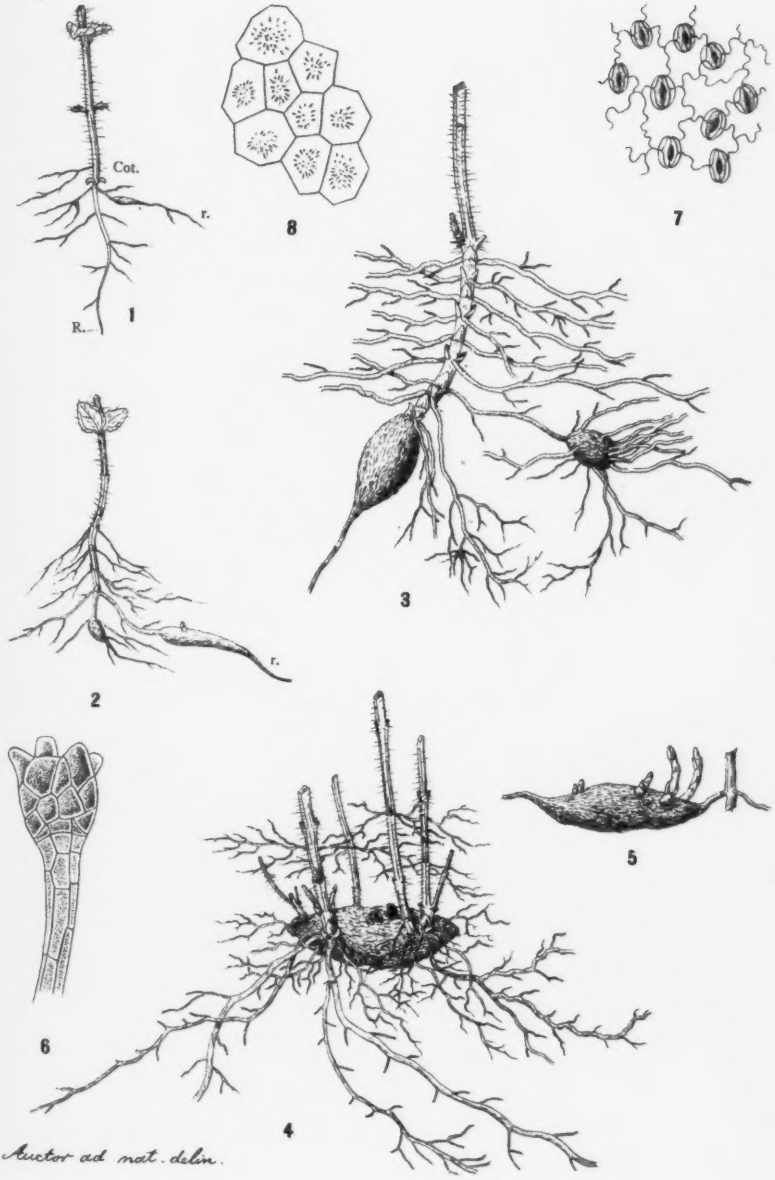
Vegetative propagation, therefore, depends upon the development of root-shoots, and, upon examining herbarium material, the same mode of propagation was found in *R. lutea* Walt., in which the roots are very long and slender, and the base of the shoot may persist for more than one season, *R. lanceolata* Walt., *R. serrulata* Nutt., and *R. ciliosa* Michx. All agree with *R. mariana* in the roots being slender and able to produce shoots. In *R. serrulata* one specimen appeared as if it was a seedling and blooming in the first season; and one specimen of *R. floridana* Nash had a tuberous root in addition to the slender, shoot-bearing root.

I believe that Rhexia must be placed with that type of plants in which the production of root-shoots is necessary to the normal development of the individual. It is a type poor in representatives, and WITTROCK enumerates species of Cirsium, Linaria, Convolvulus, Thesium, Coronilla, Epilobium, and Euphorbia as representing it. *Thladiantha dubia* Bge. doubtless belongs to this same category, according to SACHS,[7] who states that "the yearly regeneration is dependent upon root-shoots which develop from tuberous swellings of the very long and slender roots." The internal structure of the roots of this curcurbitaceous plant is described by SCOTT and BREBNER,[8] but is very different from that observed in Rhexia.

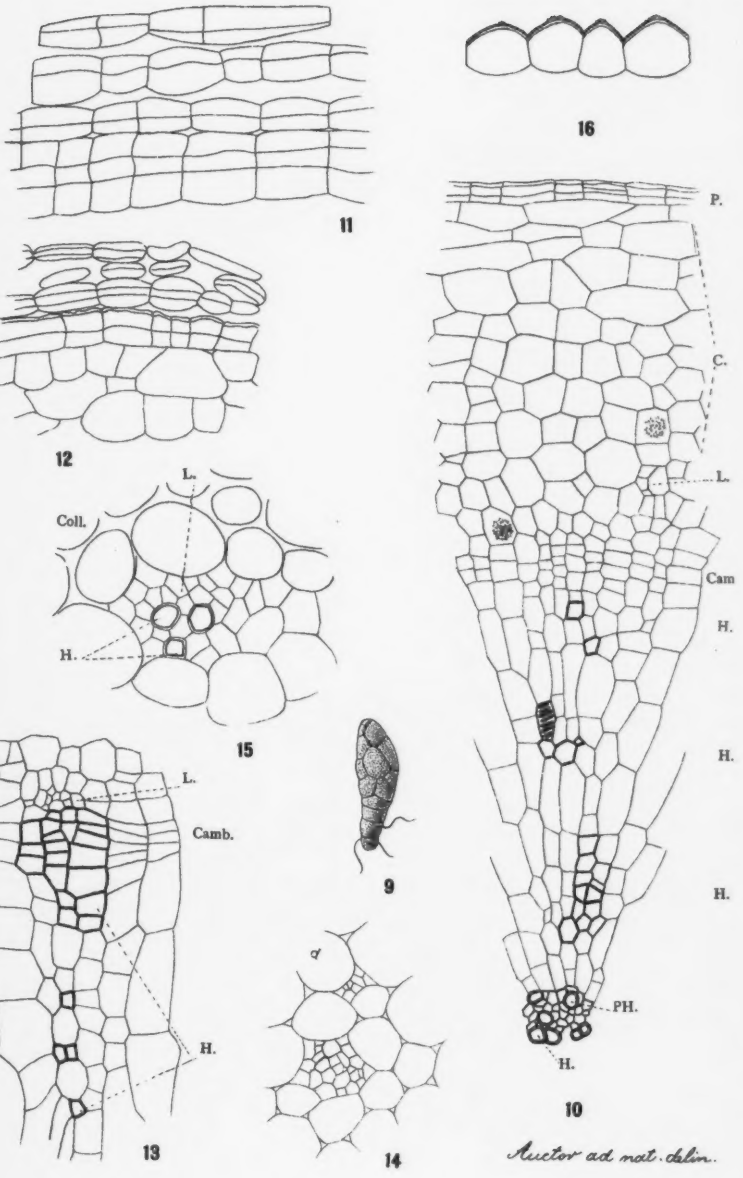
BROOKLAND, D. C.

[7] Vorlesungen über Pflanzen-Physiologie 28. Leipzig. 1882.

[8] Annals of Botany 5:273. 1890.



HOLM on RHEXIA



HOLM on RHEXIA

EXPLANATION OF PLATES I AND II

PLATE I

Rhexia virginica

FIG. 1. Seedling, showing basal internodes, cotyledons (*Cot*), primary root (*R*), and a lateral root with the tuberous swelling (*r*). ×1¾.

FIG. 2. A root-shoot developed from tuberous root of seedling; a lateral root swollen (*r*) and showing bud which will develop a shoot during next season. Natural size.

FIG. 3. The underground portions of a mature root-shoot; one of the secondary roots bears a tuber. Natural size.

FIG. 4. An old tuber with shoots. Natural size.

FIG. 5. An old tuber with buds. Natural size.

FIG. 6. Apex of a long glandular hair. ×320.

PLATE II

Rhexia mariana

Fig. 7. Epidermis of lower face of leaf. ×320.

FIG. 8. Epidermis of upper face of leaf. ×320.

Rhexia virginica

FIG. 9. Glandular hair. ×320.

FIG. 10. Transverse section of the tuberous root of *fig. 2*, showing cork (*P*), secondary cortex (*C*) with druids, leptome (*L*), cambium (*Cam*), hadrome (*H*), and protohadrome (*PH*). ×204.

FIG. 11. Cork of the same tuber. ×320.

FIG. 12. Cork of an older tuber. ×320.

FIG. 13. Transverse section of an old tuber, showing outermost part of a mestome strand; index letters as above. ×320.

FIG. 14. Central leptome strand of stem. ×320.

FIG. 15. Ventral supernumerary vein of leaf, also showing thick-walled colorless tissue (*Coll*), leptome (*L*), and hadrome (*H*). ×560.

FIG. 16. Transverse *Rhexia mariana* section of ventral epidermis of leaf. ×320.

THE SPORANGIUM OF THE OPHIOGLOSSALES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY
XCIV

L. L. BURLINGAME

(WITH PLATES III AND IV)

The sporangium of the Ophioglossales has been studied by RUSSOW ('92), GOEBEL ('80, '81), HOLTZMAN ('92), ROSTOWZEW ('92), CAMPBELL ('95, :05), BOWER ('96), CARDIFF (:05), STEVENS (:05), and BEER (:06). Of these the last three concern themselves almost wholly with the later stages of the development of the sporangium and with the development of the spores up to the time of shedding. The former investigators devoted their principal attention to the early stages of sporangial development. Of their accounts, BOWER'S is the most comprehensive and, so far as the earlier stages of Ophioglossum are concerned, the most satisfactory.

In the fall of 1906 there came into my hands, through the courtesy of Professor JOHN M. COULTER and Dr. CHARLES J. CHAMBERLAIN, some fertile spikes of *Ophioglossum reticulatum*, which had been collected by Dr. H. N. WHITFORD at Lamao, Bataan, Philippine Islands. The spikes, from plants growing at an altitude of seventy-five feet above sea level, were collected July 26, 1904, killed and fixed in chrom-acetic acid, run up through the alcohols to 70 per cent., and sent to Chicago.

With the completion of this investigation each of the three genera of Ophioglossales will have been studied throughout the entire period of development of the sporangium and spores up to the time of shedding, so that a discussion of the development of the sporangium in the group seems timely. In this discussion Ophioglossum will be made the type, with frequent comparison with the other genera. The material of *O. reticulatum* showed no stages earlier than those represented in *figs. 1* and *8*, where the wall is about three-layered, the tapetum one-layered, and the sporogenous tissue about two divisions short of the mother-cell stage. This renders it necessary to obtain the account of earlier stages from the writings of former investigators.

Earlier stages

OPHIOGLOSSUM

According to BOWER, the first indication of the regions from which sporangia are to form is the differentiation along each side of the spike of a band about two cells wide. The cells of this band are distinguished from their neighbors by a greater depth, a difference in arrangement, and differences in cell contents. He calls this the sporangiogenic band. The cells are for the most part arranged in pairs, but he does not regard this as sufficient evidence of their common origin, for he says that if the cells concerned in forming a single sporangium originate from a single cell, it must be very early in the formation of the spike. Nor did he succeed in distinguishing them at a stage so early as to enable him to trace their origin. At a slightly earlier stage, GOEBEL figures the band as only in part two cells wide. His figure seems to support his contention that the band becomes two cells wide by the longitudinal anticlinal splitting of the cells of a band but one cell wide. Some of BOWER'S figures show a far less regular arrangement of the cells in the band. In these cases, as he clearly points out, it is much less easy to refer the origin of the band to a single row of cells. It is not unlikely that the difference of opinion is due to the recognition of the band at slightly different stages in the growth of the sporangial spike, for it is not unusual for subsequent growth to displace cells so as to render their relationship to one another obscure. Whatever may be the exact mode of origin of this band, the really important point is that sporangia may arise in any part of it, though in fact they do not arise in all parts of it. It is this partial sterilization of a band potentially sporangiogenic throughout on which BOWER lays special stress.

The cells of this sporangiogenic band divide periclinally, separating a potentially primary sporogenous band from the wall cells. According to BOWER, each archesporium consists usually of a single pair of the cells of the band; where the band is three cells wide, there are three cells in the archesporium. GOEBEL, with whom CAMPBELL agrees, thinks the archesporium is unicellular. ROSTOWZEW also claims a unicellular archesporium, but RUSSOW contends that it is a cell-complex. From such divergent opinions the truth is difficult to choose, though it seems not unlikely that the band may be recognizable

while only one cell wide, and that the first division in each cell is periclinal and cuts off the potentially sporogenous cells from the ones destined to form wall and tapetum. The outer segments, according to BOWER, by repeated division add considerably to the sporogenous tissue. It is specifically stated by BOWER and ROSTOWZEW, and implied by CAMPBELL, that the tapetum arises from the outer part of the sporogenous tissue.

The species studied were *O. pendulum*, *O. vulgatum*, *O. reticulatum*, by BOWER; *O. pendulum* by CAMPBELL; and *O. vulgatum* by GOEBEL and ROSTOWZEW.

HELMINTHOSTACHYS AND BOTRYCHIUM

In *Botrychium virginianum* HOLTZMAN figures the sporangium as arising from a single cell. Writing of Helminthostachys, BOWER says: "The sporangia are similar in origin and mature structure to those of Botrychium, the essential parts being referable to a single superficial cell, of which the first periclinal division defines the sporogenous from the protective parts." In agreement with RUSSOW he thinks that in at least some species of Botrychium the archesporium may consist of more than one cell. GOEBEL and CARDIFF think the archesporium in *B. lunaria* and in *B. virginianum* is one-celled. CAMPBELL likewise thinks that the archesporium of Botrychium is a single cell and that the entire sporangium is referable in origin to this one superficial cell. He ascribes the origin of the tapetum partly to the wall and partly to the outer sporogenous tissue. GOEBEL figures it arising from the wall and sterile cells surrounding the sporogenous tissue. CARDIFF also is positive that it comes wholly from the wall and tissue outside of the sporogenous mass. In Helminthostachys BOWER has shown that the sporangium originates from a single superficial cell, that there is a single primary sporogenous cell, and that the tapetum arises from the wall.

Later stages

The preceding account brings the development to the earliest stages shown in the writer's material, the discussion of which may be taken up conveniently under three heads: the wall, the tapetum, the sporogenous tissue.

THE WALL

Owing to the conflict of opinions, the youngest material at hand was examined for evidence of the origin of tapetum and sporogenous tissue. These sporangia have a wall three cells thick, or in some cases the spindle and beginning of the wall between the middle and innermost cell. *Fig. 1* shows the next stage, where the wall is three or four cells thick. Where the wall is four cells thick and the position of the walls indicates the last one formed (wall *1* in the figure), the division is seen to have occurred in the outer cell of the three-layered wall. The fifth layer of cells also arises in a similar way by the division of the cells of the outer layer of a four-layered wall (*figs. 2* and *3*, wall *1*). This is made clearer by an examination of the cell row between the two containing five each in *fig. 2*. It may be seen that the next division will occur in the outer cell.

The order of development may be stated in the reverse order as follows: If the cell layers be numbered from within outward *1*, *2*, *3*, *4*, *5*, then *5* and *4* arose from the division of a cell which we may call *c*. In like manner *c* and *3* arose from the division of a cell *b*. As already pointed out, *1* and *2* arose from the division of a cell *a*. The tapetum is already formed and differentiated at the two-layered stage of the wall, so that two suppositions may be entertained in regard to the early history of the wall. First, *a* and *b* may have been derived from the division of a cell *w*, sister to the tapetal cell. Second, cell *a* and the tapetal cell may be sister cells derived from the division of a cell sister to *b*. Of these two suppositions, both of which assume the tapetum to have originated from the wall, the former seems the more probable. That the tapetum does arise from the wall seems to be indicated by the close similarity of its cells in shape and position to the tabular wall cells and their contrast to the polygonal sporogenous cells. In the very earliest stages observed it is difficult to distinguish tapetal cells from wall cells except by position, though the sporogenous cells are easily distinguishable in almost all cases. It is not unlikely that the first division of the cell (or perhaps cells) of the sporangiogenic band is a periclinal one and separates the primary sporogenous from the primary wall cell. A periclinal division of the latter separates the primary tapetal cell from the wall cell, subsequent divisions of which produce the five-layered wall. If this interpreta-

tion be accepted, then Ophioglossum agrees with the other genera in the origin of sporangium, wall, primary sporogenous cell, and tapetum.

This regularity of division is limited to the part of the outer wall which from its position may be inferred to have arisen from the sporangiogenic band. In other parts of the wall, not only is the division less regular, but the number of layers is greater. Furthermore, longitudinal sections show that probably this regularity is still further limited to the cells derived from the initial cell (or cells) of the sporangium.

By the time the sporogenous tissue has reached the mother-cell stage, the wall has its full number of cell layers. The further development of the wall consists of an increase in size of the hypodermal cells in the tangential plane, without any increase of thickness, and a growth and anticlinal division of the epidermal cells. In mature sporangia the epidermal cells are relatively deep and narrow, with thickened outer walls. *Fig. 7*, which shows a sporangium at time of dehiscence, may be compared with *fig. 6*, from a sporangium whose sporogenous tissue had reached the stage of young tetrads. *Fig. 7* has just half the magnification of *fig. 6*, from which it is evident that the epidermal cells have just about doubled their depth, and have in some cases decreased in width through anticlinal division. As already remarked, the hypodermal cells do not increase in thickness, but become extended laterally as the sporangium enlarges. From about the time the young spores begin to round up, they gradually lose their cell contents and become flattened as growth of the sporangium progresses. Sooner or later the walls of the inner two or three layers, and often all the hypodermal layers, disappear. In *fig. 7* portions of the walls of two layers can be traced, but in other cases the only remains consist of a line of débris between the spore-mass and the epidermis.

At the earliest stage examined, the wall cells contain considerable starch, which increases up to the time the wall has attained its full number of cell layers. After this, which is about the time the tapetal plasmodium invades the crevices of the sporogenous tissue, it gradually wastes away, until none at all is present at time of dehiscence. Its disappearance may be connected with the accumulation of starch in the tapetal plasmodium, a matter which will be discussed subsequently.

Longitudinal sections (*figs. 6* and *7*) show the method of dehiscence. About the time the young tetrads are forming, each sporangium shows a slight transverse groove. *Fig. 6* shows that this corresponds to a band of smaller thin-walled cells. The groove becomes more pronounced through the failure of the epidermal cells along it to increase in depth as fast as the neighboring ones. Finally a rupture of the sporangium occurs along this groove, probably due to strain set up by unequal growth. The spores are shed through this slit, which, so far as the material at hand indicates, seems to play a purely passive rôle in the process.

THE TAPETUM

The youngest stage of the tapetum observed is shown in *fig. 1*. That this probably originates from the first periclinal division of the primary wall cell has already been pointed out. It divides periclinally once (sometimes oftener, as shown in *fig. 5*), before the cells of the sporogenous tissue have reached the mother-cell stage. Usually no more strictly periclinal divisions occur. As the sporangium grows, the tapetal cells elongate and divide obliquely (*figs. 2, 3, 5*). *Fig. 3* presents a regional view of about one-half of a sporangium with the tapetum well developed and distinct at the left of the figure, where it may be supposed to be derived from the primary wall cell. At the right of the figure the tapetal cells are not easily distinguished from the sporogenous tissue, and their origin is not clearly indicated, though they seem rather more closely related structurally to the sporogenous cells than to the sterile cells around them on the outside. In Botrychium GOEBEL has shown that this part of the tapetum arises from the sterile cells outside the archesporium. In the present study no clear evidence for either view was obtained, owing to lack of young sporangia.

At the earliest observed stage the tapetal cells are distinguishable from the sporogenous tissue by staining reactions of the cell contents and by the position of the walls. They are less easily distinguished from the wall cells, but are on the whole slightly thicker radially. The cytoplasm is clear and highly vacuolate and so takes the stain lightly. It does not contain starch nor any form of stored food evident under the microscope. In this respect it differs from the

cytoplasm of the inner wall cells, which even at this stage contain a little starch; the outer wall cells contain considerable at this time. The tapetal cytoplasm was not observed to contain any starch until after the walls have broken down. The walls themselves are from the first very delicate, but are sharp and distinct when properly stained, so that there can be no mistake as to their presence. In this respect Ophioglossum seems to differ from *B. virginianum*, whose tapetal cells, as described by STEVENS, are delimited by a plasmatic membrane only. CARDIFF, who studied the same species, does not mention this circumstance, and BEER calls attention to the fact that it is not true in Helminthostachys. The nuclei are about 20 μ in diameter, but vary somewhat both in size and shape. They stain rather deeply and are generally not otherwise distinguishable from the nuclei of the wall cells.

When the mother-cell stage is reached, the tapetum is at least two-layered throughout and its walls have become very thin and about this time have begun to break down. The cytoplasm of the neighboring tapetal cells mingles and forms a plasmodium, as described by CARDIFF for Botrychium. In Botrychium as well as in Helminthostachys the tapetal cells are elongated radially, while in Ophioglossum they are flat and tabular, the radial diameter being the shortest. Ophioglossum also differs from the other genera in having usually only two layers of tapetal cells, whereas they have from two to four layers. It seems likely that the walls break down sooner in Ophioglossum than in the other genera. This is probably correlated with the fewer layers and with the fact that in Botrychium the cells are frequently binucleate. Too few preparations at just the stage when the walls are breaking down were seen to make sure that binucleate cells do not occur, but none were seen.

BOWER and others have stated that at this time some of the mother cells break down and contribute to the tapetal plasmodium. This is denied by CARDIFF for Botrychium, and by BEER for Helminthostachys. No certain case of such a contribution was observed in Ophioglossum, but the number of preparations of just this age observed were too few to justify the statement that it never occurs. Mother cells which did not appear normal were found in two or three preparations, but in each case they occurred in sporangia that were

in other respects clearly abnormal, suffering from external injury to the wall in one case, an abortion of the inner wall cells and neighboring sporogenous tissue in another, etc. In sporangia apparently normal in all respects, a few of which were observed, no trace of such a contribution was found. It is not unlikely, therefore, that this genus agrees with the other two in this feature also.

At the time the plasmodium begins to penetrate among the crevices of the sporogenous tissue they are rather small, and the movement is therefore gradual. Hence the deeper parts of the crevices are empty of cytoplasm. By the time the sporangium has reached the stage shown in *fig. 4*, the plasmodium completely fills every crevice, showing that the cytoplasm increases in volume after the breaking-down of the walls. The nuclei do not at first penetrate among the blocks of sporogenous tissue on account of the narrowness of the clefts, but later as these enlarge they become distributed throughout the sporangium.

The structure of the plasmodial cytoplasm is at first coarser and stains more deeply than did that of the tapetal cells before the breaking-down of the walls. It has a ropy appearance and contains many large granules. It does not seem to be definitely vacuolate, but among the beaded threads there seems to be a fine hyaline sap-like substance (*fig. 4*). At this time the plasmodium does not contain any starch, which, however, soon appears and continues to increase until the plasmodium is fairly crowded with it (*fig. 16*). The stringy cytoplasm becomes denser during the formation of the starch and finely vacuolate. The maximum density and starch content is reached about the time the tetrads are complete. From this time on the starch grains become fewer and fewer (*fig. 25*) until they have entirely disappeared. By this time the cytoplasm, which has been growing more and more vacuolate, has nearly all disappeared. The last traces of it are to be seen along the old plasmatic membranes, which may often be traced long after the more fluid parts have disappeared. When the spores are of the age shown in *figs. 26* and *27* all traces of it are usually lost.

The nuclei of the plasmodium exhibit frequent changes of form in order to accommodate themselves to the spaces which they are compelled to occupy, or into which they perhaps force themselves. Where one is found in a narrow cleft it has the appearance of being about to

divide amitotically. No actual case of such amitosis, however, has been observed in any stage of the tapetum. No mitotic figures have been observed in the plasmodium either, though the nuclei seem somewhat more abundant in the later stages. The sporangia vary so much in size and consequently in number of nuclei found at any stage that it is not possible to say with confidence that the nuclei increase after the walls of the tapetum have disappeared. That the nuclei do not move about passively in currents of cytoplasm seems to be indicated by the fact that where one is apparently just entering a crevice it is always drawn out to a point, which stains more deeply with chromatin stains than do the other parts of the nucleus. BEER has called attention to similar appearances of the nuclei of Helminthostachys. The average size of the nuclei does not differ materially in older stages from that of the earlier ones. There is considerable individual variation of both size and shape at any stage. CARDIFF has pointed out that the nuclei of the plasmodium of Botrychium increase fourfold in size; but BEER has found that this is not the case in Helminthostachys. The structure of the nucleus, however, does undergo considerable change. A reference to *fig. 16* shows the tendency of the chromatic material in the later phases of development to aggregate itself into elongated masses, whose long axes are directed lengthwise of the nuclei. These masses are much fewer than the chromosomes. A similar condition is shown in BEER'S figures of Helminthostachys. When the nuclear membrane breaks down, these masses are spilled out and persist for some time. The nuclei persist longer than the cytoplasm; it is not uncommon to find one or more of them in sporangia from which the spores are just about ready to be shed.

THE SPOROGENOUS TISSUE

The earliest stage of the sporogenous tissue observed is shown in *fig. 8*. At this time it probably lacks about two divisions of the mother-cell stage. The cells are squarish or polygonal, and about 25 μ in diameter. The nuclei, which vary slightly, average about 18 μ in diameter. The cytoplasm is delicately reticulated and does not stain very intensely. It seems to be made up of a network of delicately beaded strands, the meshes of which are filled with a hyaline ground-substance which stains very lightly or not at all. The

wall is very thin but distinct. STEVENS reports the sporogenous cells of Botrychium as being devoid of a wall. The cells at this stage, in the living condition, are probably still attached to one another so as to form a compact tissue. In stained preparations clefts appear among groups of cells. It is probable that some or all of these clefts are the effects of contraction in the preparation of the material. The nucleus is usually quite round and is surrounded by a sharply defined nuclear membrane. The nuclear network is not very unlike that of the cytoplasm, except that the beaded strands are larger, stain more deeply, and present many more loose ends. From one to six nucleoli are usually present in each nucleus.

Whether the blocking of the sporogenous tissue begins as early as the stage shown in *fig. 8* or not, there is no doubt that it is clearly present after the next division of the sporogenous cells. Such a condition is shown in *fig. 2*, where the sporogenous tissue is seen to be broken up into irregular blocks, and to be free from the tapetal cells, which are beginning to separate from one another. *Fig. 3* presents a view of a slightly more advanced state of blocking. The irregularity of Ophioglossum is in sharp contrast to the almost diagrammatically regular blocking of the sporogenous tissue of Botrychium. According to CARDIFF, the division of the sporogenous tissue in that genus is in three planes at right angles, and very regular and uniform in all parts of the sporangium. He suggests that these walls break down in the order of their formation, with the result that the tissue breaks up into regular rectangular blocks. His figures lend considerable support to this view, and there seems to be no reason for doubting it. The divisions in the sporogenous tissue of Ophioglossum are far less regular, and this may be the cause of the irregularity in the blocking. BEER notes that the process in Helminthostachys is less regular than in Botrychium. It is far more regular, however, than in Ophioglossum. An exceptionally regular case is shown in *fig. 5*.

Correlated with the question of blocking is that of the division of the sporogenous cells. In Botrychium, and to a less degree in Helminthostachys, division is simultaneous in the sporogenous tissue up to the time of blocking. After the blocks become independent, the cells of each block divide simultaneously, but different blocks may develop at different rates. Even so early as the stage shown

in *figs.* 1 and 8, the cells do not develop at the same rate nor do they divide simultaneously. That there is some coordination, however, is shown by the fact that figures do not usually occur isolated, but in small groups. Cells in the synapsis stage were noted in some sporangia when other cells seemed not to have reached the mother-cell stage. Oddly enough, however, most or all of the cells come out of the synapsis at about the same time. This seems to be the only point in the development at which the cells are all at approximately the same stage. Each cell seems to develop independently of the others in the majority of cases, although there are not lacking frequent cases where several cells lying side by side are approximately in the same phase. The most striking examples of this, are groups of cells in the late prophase often found among young tetrads or even in sporangia, where the young spores have become separated and have rounded off. *Fig.* 9 gives a view of a mother cell in synapsis (the synaptic knot has been simplified in drawing for the sake of clearness). The cells at this stage are oblong and about 28×32 μ. The cell shown in the drawing is one of the largest, being 27×40 μ. The walls are at this time very thin and the cells have either separated from one another entirely or into small groups. The walls are so thin that it was sometimes impossible to find them, though it was always possible to find other cells younger, of the same age, and older, which showed the wall, so that it seems likely that all of them really have walls. It has already been noted that STEVENS did not find them in *B. virginianum* at certain stages. CARDIFF shows in his figures what seem to be walls, but makes no text comment. BEER is emphatic in his assertion that they are present at all stages in Helminthostachys. Recalling their exceeding thinness and the difficulty of staining them, one must accept with considerable reserve the statement that they are absent at any stage. However, when one considers that most of the walls disappear in the breaking-up of the sporogenous tissue, it does not seem altogether improbable that they may totally disappear in some cases. It may be added that the writer has been able to find them in preparations of *B. virginianum* of the same age. The preparations examined do not cover all stages, and he is therefore not able to say positively that they are always present.

The cytoplasm is very like that already described for the earlier phases of development, except that it is somewhat denser, especially around the nucleus. The synaptic knot is usually so closely massed that the course of the individual thread or threads cannot be followed for any great distance. The figure was drawn from one of the most diffuse knots in a section 10 μ thick. The chromatin seems to consist of a single row of granules strung along a continuous much tangled thread. The thread could not be determined to be continuous by direct observation, but this is made probable by the fact that there are no loose ends protruding from the knot on the uncut side. The same is true of other knots. In the early stages the thread is extraordinarily long and thin, long enough nearly to fill the nucleus with its tangled folds, and not more than 0.25–0.5 μ thick. The thread breaks up into very long slender chromosomes, which may have almost any shape from nearly straight to intricately bent and twisted.

A somewhat later stage is shown in *fig. 10*, where the chromosomes have shortened by one-half or more and have become correspondingly thicker. In the figure no attempt has been made to represent all the chromosomes in the nucleus, but only those whose shape and position could be made out clearly. The drawing was made from a section in which the knife has passed almost exactly through the center of the nucleus, and the cut side of the nucleus lies up. This gives a clearer field, but has the disadvantage that some of the chromosomes are cut in two. More than sixty chromosomes are shown in the drawing of this half-nucleus. Attempts were made to count the chromosomes at this stage, in the stage shown in the next figure, in polar views of the second division, and elsewhere, but without success. The number exceeds 100, but probably does not reach 120 for the gametophytic tissues. The chromosomes continue shortening and thickening until they reach the shape and size shown in *fig. 11*. The nuclei have their maximum size at about this stage, being 23–24 μ in diameter.

During the development of the chromosomes the wall remains thin and stains lightly, as in previous stages. The cytoplasm, however, has increased in density somewhat and has become more finely vacuolate. There appear in it about this time certain oval bodies staining very intensely with Haidenhain's iron-alum and haematoxylon, but

lightly with most other stains. The staining reactions make it improbable that they are starch or chromatic material or any of the more usual constituents of cells. They are probably some sort of stored-food material, since they disappear slowly at a later stage, when such stored foods are being used. They appear in all stages following this up to about that shown in *fig. 23*. Their number and size in different cells varies greatly, in some being absent. Some of them are shown in *figs. 11–21*. They are usually much more numerous than shown in the figures, which show only those visible in a single optical focus, or even fewer than that in *figs. 17–20*. They often line up along the central region of the spindle of the first division, or along the subsequent wall (when one forms) in such masses as to obscure the details of cell structure entirely in that region. The cytoplasm itself grows denser and denser as the time of division approaches; *fig. 11* shows it at about its maximum. *Figs. 11* and *13* show two different aspects of the cytoplasm of dividing cells, the former being the common one. The beaded threads shown in *fig. 12* are very characteristic of the cytoplasm of the cells during maiosis.

The spindle originates as a weft of kinoplasmic fibers around the nucleus. The fibers then form a number of poles such as those shown in *fig. 11*. Three of these poles become prominent and the others disappear. The tripolar stage passes into the bipolar by the further elongation of two poles and the breaking-up of the third (*fig. 12*). *Fig. 13* presents a surface view of the mature, sharply pointed, bipolar spindle. The chromatic mass (simplified in the figure) proved too dense and the chromosomes too small and numerous to make any trustworthy observations on the method of chromosome splitting. The chromosomes usually pass to the poles in a more or less straggling manner, though sometimes they advance in regular lines. Reaching the poles they may or may not organize a resting nucleus; when the second division follows very soon they probably do not. *Fig. 14* shows the beginning of the formation of the resting network before the spindle has disappeared. *Fig. 15* shows the chromosomes still free after the formation of a wall between the daughter nuclei. In *fig. 16*, from which the spindle fibers have not entirely vanished, the daughter nuclei are in the resting condition.

After the first division, a wall may (*figs. 15, 16, 19, 20*) or may

not (*figs. 14, 17, 18, 21*) form before the next division. Probably this depends on the rapidity with which the second division follows the first, and perhaps the usual thing is for the second division to occur when the daughter nuclei have reached about the stage shown in *fig. 14*. At this time the chromosomes usually have not formed a resting network, and the central part of the first spindle still persists. If division occurs at this stage, the resulting figure usually resembles *figs. 17* and *21*. The majority of the spores are of the tetrahedral type. In this region of the cell, whether the wall forms or not, there is always a noticeable collection of fine granular cytoplasm which is sharply distinguishable from the less dense parts containing the beaded strands already mentioned. Within this zone most or all of the food granules are found; they are often so numerous and so densely crowded as to make it difficult or impossible to tell whether a wall is present or not.

Early stages in the development of the spindle of the second division were not observed. Metaphases are shown in *figs. 17* and *18*, and anaphases in *figs. 19* and *20*. All the spindles with the exception of the type shown in *fig. 18* are sharply bipolar, though somewhat blunter than those of the first division. The spindle fibers are markedly different from those of the first division; instead of a loose bundle of very fine fibers, the fibers are heavy and coarsely beaded. All the spindles arranged as in *fig. 18* have their apices truncated. *Fig. 19* shows one spindle in the linear position, the other not; in this type no truncated spindles were observed. *Fig. 20* shows the bilateral arrangement; no other figure with this arrangement was observed, though one case of bilateral spores was noted (*fig. 25*). *Figs. 17–20* are arranged in the order of frequency of their occurrence in the preparations. No spores were found which could be referred to the types of division shown in *figs. 18* and *19*, which is singular, considering the frequency of this type of arrangement at time of division; they were probably present, but were not recognized. In *fig. 20* the chromosomes seem to be passing into the network characteristic of resting nuclei even before reaching the poles of the spindle, and long before any nuclear membrane has formed.

During maiosis the wall of the mother cell still persists and is very noticeable, owing to its separation from the contents, for which it

seems ordinarily to be far too large. Such a wall, in contact with the contents at one point only, is shown in *fig. 15*. This wall persists around the tetrad until late in the development of the spores. The wall with its contents lies in a vacuole of the tapetal cytoplasm. The vacuole is limited by a plasmatic membrane often more prominent than the wall of the mother cell within it. Where the two are in contact they are distinct, showing that the vacuole is a real one and is not merely the effect of shrinkage in preparing the material. I was not able to determine just how long the old wall persists, but it was found (at least parts of it) around spores as old as those shown in *fig. 27*.

The beginning of the tetrad walls is shown in *fig. 21* and the completed wall in *fig. 22*. It is formed partly on the primary division spindles of the second division and partly on secondary spindle-like structures which arise between the nuclei which are not sisters. Of the six walls which meet in the middle and separate the young spores, two form on the primary spindles and four on the secondary ones. At the same time a wall forms on the outer or convex side of the spores. Whether this wall joins directly to the tetrad wall or continues around inside of it could not be determined. BEER describes this tetrad wall as uniting with the wall which surrounds the tetrad, thus forming a structure with four compartments, each of which contains a spore. It is certain that it does not do so in Ophioglossum, owing to the fact that the mother-cell wall is not at that time in contact with the spores.

The cytoplasm of the young spores is dense and granular. The resting nucleus has a rather coarse network, with the chromatin scattered over it in rather large masses (*fig. 22*). As the spores grow older they break apart, the walls thicken, and the cytoplasm becomes vacuolate. The progressive vacuolation of the cytoplasm seems to be connected with the corresponding rapid thickening of the young spore coats. The relation between the two is brought out in *figs. 22–26*. During this period of thickening of the spore walls the cytoplasm of the tapetal plasmodium also becomes more vacuolate. A decrease of its starch accompanies *pari passu* the vacuolation of the plasmodium. The degree of the collapsing action of the killing and fixing agents on the young spores affords a graphic though not very

accurate means of estimating the decreasing density of their cytoplasm. At about the time the spore coats attain a sufficient rigidity to resist collapse, the cytoplasm begins to increase in density (*fig. 27*). Soon afterward (*fig. 28*) starch makes its appearance in the spore. About the same time the spore coat can be seen to consist of an exospore and an endospore. As the spore becomes older the two regions of the wall become more distinct and the exospore increases considerably in thickness. In the mature spore there are two cytoplasmic zones, an outer less dense one and an inner dense one filled with starch.

Whether the endospore originates as a separate membrane or whether it is merely the inner part of an originally homogeneous membrane which has differentiated into two could not be determined in Ophioglossum. Appearances, however, favor the latter view. Perhaps the strongest evidence in its favor is the fact that though the endospore is less distinct when first distinguishable, it is just as thick or even thicker than in the mature spore. Somewhat various opinions have been held concerning the exact method of exospore formation. DEBARY (**'64**) states that the exospore of certain Ascomycetes is laid down from the epiplasm, which might be thought to correspond roughly with the tapetal plasmodium in respect to the part either might play in the formation of the outer coat of the spore. The fact that the entire tetrad is surrounded for a considerable time by the persistent mother-cell wall, and that the whole lies in a vacuole, would seem to render this hypothesis untenable in respect to Ophioglossum. FITTING(**:00**), BEER (**:04**), and others maintain that the exospore, even though separated from the endospore and the protoplast by a space containing no protoplasm, can be built up by its activities. Miss LYON (**:05**) has shown that the two coats in Selaginella are formed by the differentiation of a single thick homogeneous gelatinous membrane. The exospore according to this view is simply the outer part in which has been deposited dense granular material; the roughness is due to the irregular way in which the material is deposited; and the endospore is the part in which no such deposits are made. This explanation seems to harmonize with the facts in Ophioglossum. Miss LYON points out that the membranes of Selaginella are not separated and suggests that where they seem so it is due to action of the reagents used in preparing the material. There is

no separation in Ophioglossum, and in consequence no necessity for action at a distance greater than the thickness of the spore coat. The supposed semi-fluid character of the membrane at the time of its formation would reduce this difficulty to a minimum.

Interesting irregularities occur in the development of the sporogenous tissue. It has already been pointed out that the mother cells may develop at very different rates. *Fig. 11*, for example, was drawn from a sporangium containing young tetrads and various stages of the first and second divisions. Still more interesting irregularities are shown in *figs. 30–33*. *Fig. 30* shows an enlarged mother cell in the metaphase of the first division; it was found among spores slightly younger than the one shown in *fig. 27*, and its wall is indistinguishable from those of the spores among which it lies. This figure afforded the best opportunity observed for obtaining an approximate count of the chromosomes. Owing to the way in which they are scattered, most of them are sufficiently free from their neighbors to be identified and counted; 102 are shown in the drawing, which represents about one-half the entire figure. About as many more could be counted in the sections adjacent to this on each side, thus making the gametophyte number somewhat over 100. *Fig. 33* shows three spores within a common exospore, one of which is plainly degenerating; no trace of the fourth member of the tetrad could be found. Such a structure could arise from the one just discussed by the division of one of the daughter nuclei and each of the three resulting nuclei and its accompanying cytoplasm surrounding itself with a wall. *Fig. 31* shows a body consisting of eight cells, with thin outer wall and very delicate division walls. The cytoplasm is dense, finely reticulated, and crowded with starch, which is also abundant in the tapetal plasmodium around it, though not found in the young spores in the same sporangium nor in any spores until they are much nearer maturity. This might suggest that the normal young spores are poor in cytoplasm and stored food because they use it to build their rapidly thickening walls. Though varying in size the nuclei are all large, being about the size of the entire young spores in the same sporangium. With Flemming's triple stain they stain uniformly with the gentian. *Fig. 32* represents another structure of the same sort but with twenty nuclei, two of which are very large. These bodies have probably arisen from

the germination of the members of a tetrad which failed to separate in the usual way. Possibly the lack of a thick wall around the young tetrad is responsible for the continued growth; at any rate, no indication of the germination of ordinary thick-walled spores was observed, unless *fig. 30* be interpreted as such. Not unlike this is the production of more than four microspores in some angiosperms, but so far as I am aware similar phenomena have been reported in only one homosporous plant. In an account of the germination of the oöspore of Coleochaete, ALLEN shows that reduction takes place with the first division; the spores thus produced germinate and cooperate to produce a multicellular mass of gametophytic tissue. Each cell of this mass of tissue eventually produces a zoospore which gives rise to the ordinary vegetative phase of the plant. WILLE has shown (**'86**) that in certain angiosperms by a suppression of the second maiotic division a mother cell may give rise to two microspores only instead of the usual four. Since in at least the majority of plants the unlike elements of the bivalent chromosomes are separated in the first division of the mother cell, there seems to the writer no valid reason, other than that imposed by the available food supply and the ordinarily thick walls, why any number of spores more than two may not arise from a mother cell. In a discussion of many-spored asci OVERTON (**:06**) has recently shown that the number of divisions in the ascus is immaterial so far as the essential nature of the resulting spores is concerned. In this connection the recent experimental work of NĚMEC (**:06**) is of considerable interest; by subjecting staminate flowers to the action of chloroform vapor he secured pathological conditions closely paralleling those herein described. His attempts to germinate these bodies should yield interesting results.

SUMMARY AND COMPARISON

The following tabular form of statement will serve to contrast compactly the three genera of Ophioglossales as to their sporangia.

Ophioglossum	*Helminthostachys*	*Botrychium*
1. Outer wall and corresponding parts of the tapetum and the sporogenous tissue from one or two superficial cells of a sporangi-	The essential parts from a single superficial cell.	Like preceding but smaller contribution of neighboring cells.

Ophioglossum	*Helminthostachys*	*Botrychium*
ogenic band; remainder of sporangium formed by the cells of the adjacent sterile tissue.		
2. Freely exposed outer wall 5-layered and from primary wall cell (or cells).	Similar to the preceding in origin and development, except that in most species the entire wall so arises.	Like preceding but still further restricted to a single cell in origin.
3. Sporangia sunken.	Sporangia intermediate.	Sporangia generally short-stalked.
4. Dehiscence by transverse slit.	By longitudinal slit.	By transverse slit.
5. One to three primary sporogenous cells.	One primary sporogenous cell.	One primary sporogenous cell.
6. Tapetum unusually two-layered and its cells tangentially elongated.	Cells radially elongated, otherwise like preceding.	Same as preceding except that there are two to four layers of radially elongated cells.
7. Nuclei of tapetal plasmodium probably do not increase in number.	Amitosis of these nuclei probably occurs.	Amitosis usual and frequent.
8. Nuclei of plasmodium do not increase in size.	No increase in size.	Nuclei increase to four times their original size.
9. Tapetal nuclei persist up to near time of dehiscence.	More persistent than in preceding.	Less persistent than other genera.
10. Sporogenous tissue separates into irregular masses.	Blocking much more regular.	Blocking extremely regular.
11. Division of sporogenous tissue up to mother cells not wholly simultaneous.		Simultaneous division the rule.
12. Maiosis in different mother cells varies widely in time and in arrangement of spindles.	Maiosis in each mother-cell independent.	Division simultaneous in each block of cells.
13. Spindle extra-nuclear.		Spindle intra-nuclear.
14. Chromosomes 100 to 120 after reduction.	Reduced number 40 to 60.	No data.
15. Spindles in all possible positions with reference to one another.		"Either in same plane or perpendicular to one another."

Ophioglossum	*Helminthostachys*	*Botrychium*
16. Wall formed or not after first maiotic division.	Special tetrad wall incloses each spore in a separate compartment.	No wall formed.
17. Tetrads for a long time inclosed within persistent mother-cell wall.		Tetrad inclosed within persistent wall of mother cell.

The features common to the genera are: (1) the breaking-down of the inner layers of the wall, (2) the penetration of a plasmodium, derived from the tapetal cells, among the sporogenous cells, (3) the failure of the mother cells to contribute to the tapetal plasmodium, (4) the formation of a resting nucleus after the first maiotic division, (5) the appearance in the spore-plasm of a phase of decreasing density followed by one of increasing density, the former possibly connected with the rapid growth of the young spore coat (no data from Botrychium), (6) the spores rich in starch, (7) the tetrads in vacuoles of the plasmodium.

This investigation was carried on under the direction of Professor JOHN M. COULTER and Professor CHARLES J. CHAMBERLAIN.

THE UNIVERSITY OF CHICAGO

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EXPLANATION OF PLATES III AND IV

All figures were drawn with the aid of a Bausch and Lomb camera and Zeiss apochromatic objectives and compensating oculars, and reduced one-half. *Figs. 1–7* and *fig. 32* were drawn under a low-power dry lens, the others under 1.5^{mm} immersion.

PLATE III

FIG. 1. Wall of three or four layers; last wall formed indicated by *1;* tapetum one-layered except at left. ×300.

FIG. 2. Wall four- or five-layered; tapetum two-layered; sporogenous tissue beginning to break up into irregular blocks and to separate from the tapetum. ×300.

FIG. 3. General view of about one-half of a sporangium, showing four or five layers in the wall; two-layered tapetum; sporogenous tissue broken up into irregular blocks; the regular two-layered tapetum is restricted to the left of the figure; in the remaining part it is difficult to distinguish it from the sporogenous tissue; *1* is last wall formed. ×123.

FIG. 4. Tapetal plasmodium penetrating among blocks of sporogenous tissue; tapetal nuclei mainly around periphery; blocks of sporogenous tissue quite irregular. ×300.

FIG. 5. An unusually thick tapetum and more regular blocks of sporogenous cells, somewhat older than those of *fig. 4*. ×300.

FIG. 6. Longitudinal section of wall; tetrad stage of sporogenous tissue; to show early indications of formation of slit for dehiscence. ×300.

FIG. 7. Mature wall showing longitudinal section through line of dehiscence; inner cell layers broken down. ×123.

FIG. 8. A sporogenous cell lacking about two divisions of the mother cell; from sporangium with a three-layered wall. ×900.

FIG. 9. Synapsis of mother cell; synaptic knot has been simplified in drawing for the sake of clearness. ×900.

FIG. 10. Prophase of first division; chromosomes about one-half the length of their earliest stage; food granules in cytoplasm. ×1200.

FIG. 11. Origin of spindle; chromosomes in mature form, many of them seem paired; food granules in cytoplasm; cell appears small because it is not drawn in median optical section. ×900.

FIG. 12. Spindle passing from tripolar to the bipolar stage; food granules and beaded kinoplasmic threads prominent. ×900.

FIG. 13. Surface view of mature spindle at metaphase; cytoplasm unusually free from food granules and kinoplasmic threads. ×900.

FIG. 14. Telophase of first division; nuclear membrane formed and chromosomes mostly free from one another; middle region of cell occupied by persistent spindle fibers, food granules, and denser cytoplasm; old wall becoming freed from contents. ×900.

FIG. 15. Later telophase; a wall has formed, along which the food granules tend to line up; mother-cell wall almost entirely free from contents; chromosomes quite free from one another. ×900.

FIG. 16. Part of tapetal plasmodium containing much starch; tapetal nuclei show the aggregation of chromatic material into elongated masses, far fewer than the chromosomes; mother cell after first division, with a wall and daughter nuclei in resting condition; the whole lies in a vacuole of the plasmodium; old mother-cell wall surrounds it, but is not in contact with it and is not shown in the drawing. ×900.

PLATE IV

FIG. 17. Usual position of spindle with no wall between, will result in tetrahedral arrangement of spores; note difference between first and second spindles; many chromosomes left out for clearness. ×900.

FIG. 18. Linear arrangement of spindle; spindle fibers do not come to a point; as in all cases, granular cytoplasm and food granules collect in middle region, whether a wall is present or not. ×900.

FIG. 19. Combination of linear and bilateral arrangement; linear spindle pointed, though this was never observed where both spindles are placed linearly; a wall in this and next figure. ×900.

FIG. 20. Bilateral arrangement of spore nuclei; chromosomes already fusing before reaching the poles. ×900.

FIG. 21. Telophase and earliest indication of tetrad walls. ×900.

FIG. 22. Young tetrad with dense cytoplasm and thin wall. ×900.

FIGS. 23, 24. Progressive vacuolation of spore-plasm and correlated thickening of spore wall. ×900.

FIG. 25. Depletion of tapetal plasmodium and increase of its vacuoles; bilateral spores shown within vacuole. ×900.

FIG. 26. Maximum depletion of spore-plasm; spore walls rather thick and probably gelatinous, collapsing under the action of the reagents. ×900.

FIG. 27. Spore wall rigid enough to resist collapsing action of reagents; wall beginning to be slightly denser and rougher. ×900.

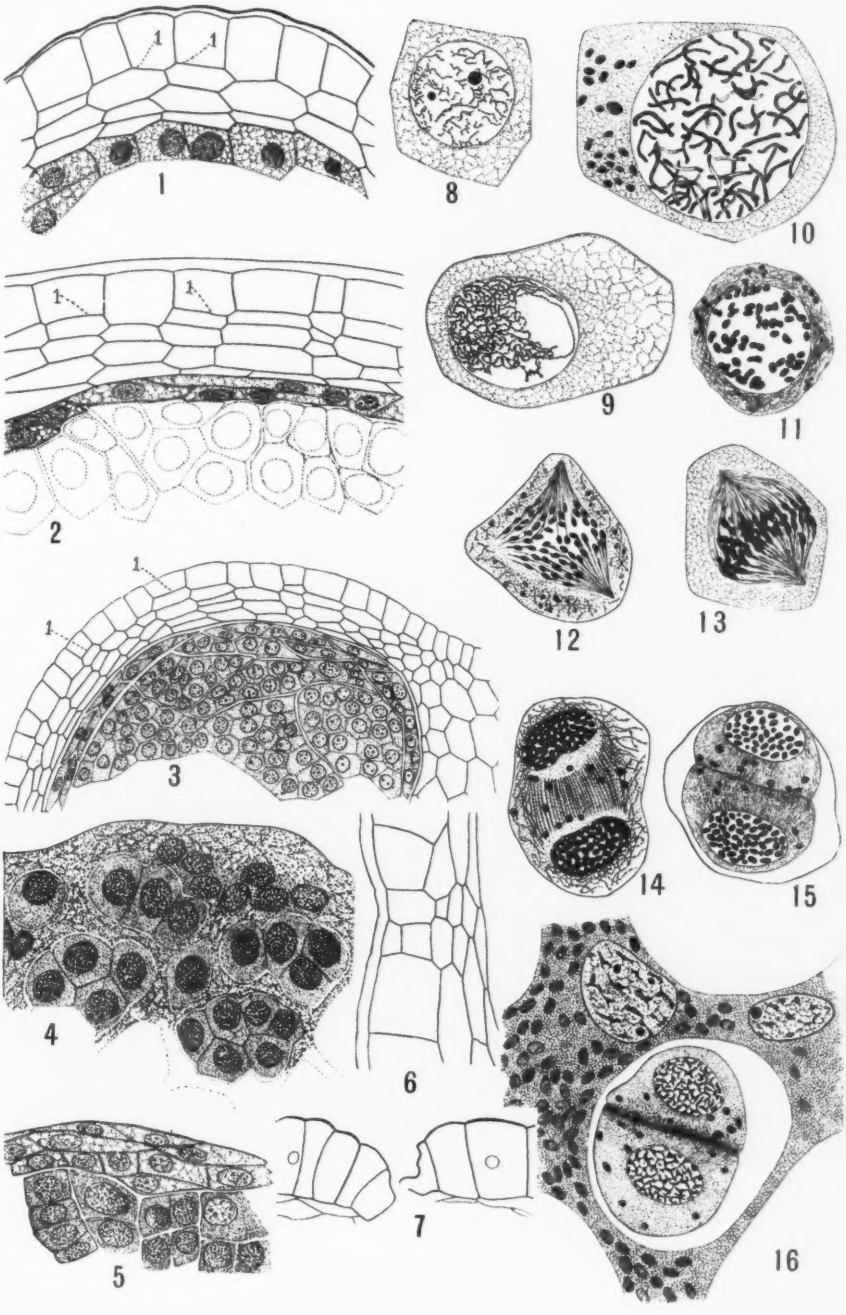
FIG. 28. Spore coat distinctly differentiated into two layers; the cytoplasm much denser and showing very small starch grains. ×900.

FIG. 29. Mature spore with thick wall and abundant contents; starch very abundant; intine penetrates exine at tip of spore. ×900.

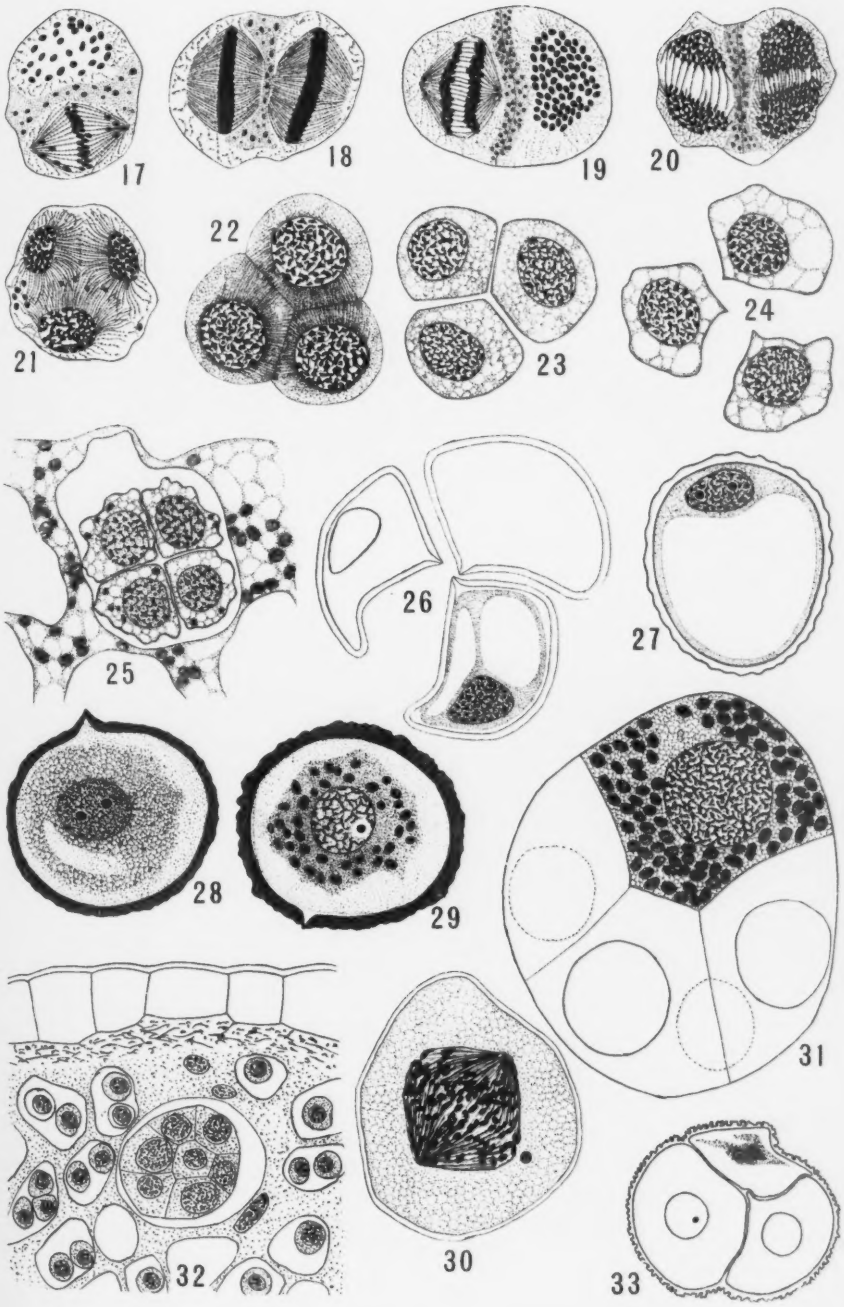
FIG. 30. Much delayed division of a mother cell, spore-coat-like wall surrounding it. ×800.

FIGS. 31, 32. Multicellular gametophytic (presumably) masses of cells, which have arisen through the cooperative germination of the members of a tetrad which failed to separate in the ordinary way. ×233.

FIG. 33. Three of the four spores of a tetrad within a common exospore. ×600



BURLINGAME on OPHIOGLOSSALES



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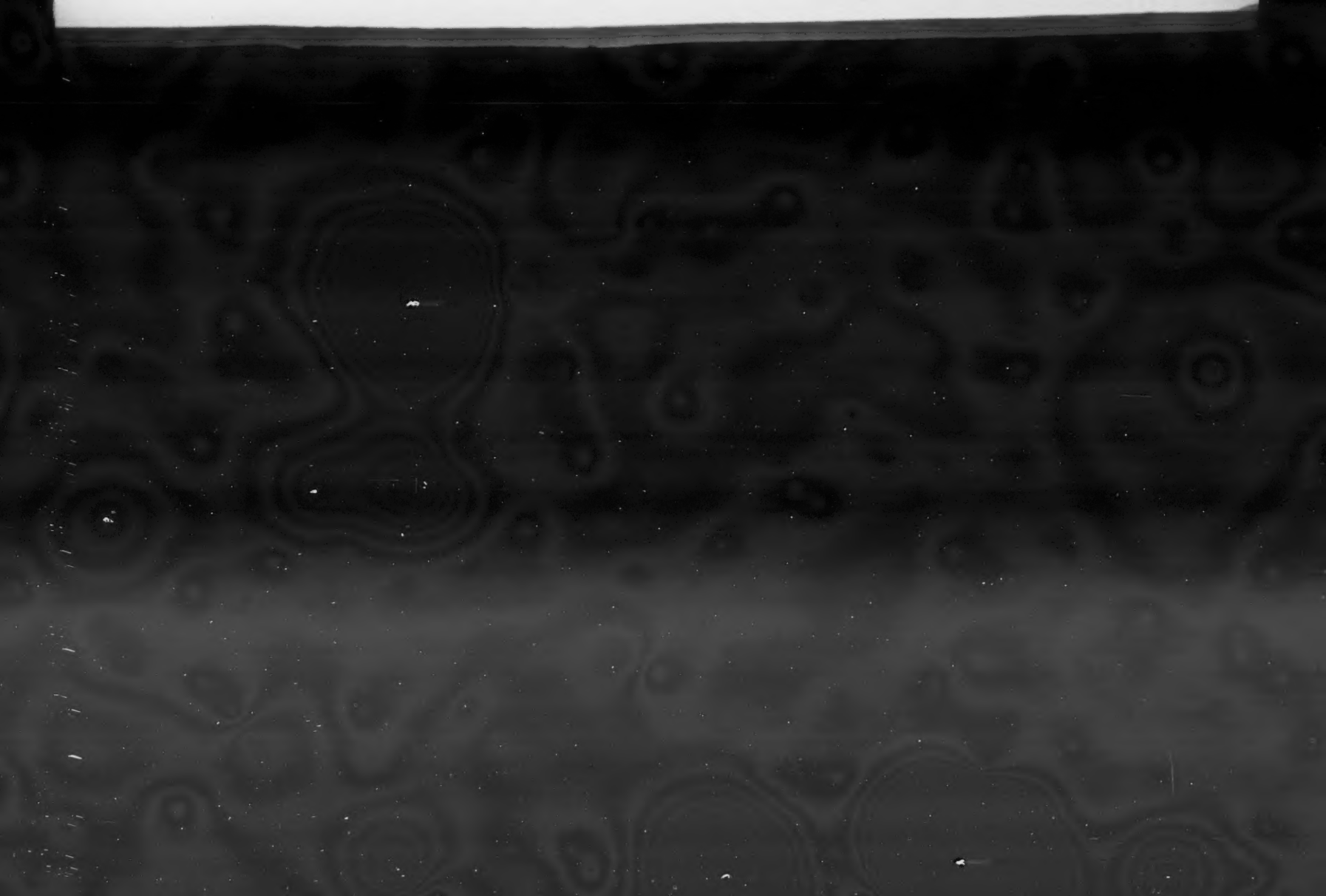

THE ROOTS OF LYCOPODIUM PITHYOIDES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XCV

ALMA G. STOKEY

(WITH PLATES V AND VI AND ONE FIGURE)

Lycopodium pithyoides Schlecht. & Chamisso is a subtropical form of rare occurrence, but, according to UNDERWOOD and LLOYD, of unexpectedly wide distribution. In spite of its striking and unmistakable appearance, it has been confused with other forms, such as *L. dichotomum*.

The material which formed the basis of this investigation was collected in September 1906 near Jalapa, Mexico, by Professor BARNES, Dr. CHAMBERLAIN, and Dr. LAND, of the University of Chicago. They found in that region only one specimen, which they secured and which is now growing in the greenhouse of the University. This plant, or rather clump of plants, was growing as an epiphyte on the trunk of a recently fallen tree.

This species of Lycopodium attains a size unusual in the genus. The stem is dichotomously branched, erect, and rigid, with a diameter of 6 to 10^{mm}; and the slender dark green leaves are 2.5 to 3.5^{cm} long. The fact that practically every leaf is a sporophyll indicates that this form is of relatively primitive character. The length of its leaves, the stoutness and rigidity of its stem, and its unusual size give it such a shrub-like aspect that SCHLECTENDAL and CHAMISSO (**10**) in the original description very aptly compared it to a young *Pinus silvestris*.

A cross-section of the stem, however, is much more striking than the general habit. The stele, which in structure and arrangement is similar to that of *L. lucidulum* or *L. Selago* is comparatively small, being about one-tenth the diameter of the stem. The conspicuous feature of the stem is not the central stele, but the numerous smaller heavily sheathed steles which surround it (*fig. 1*). These are the "inner roots" of STRASBURGER (**11**). The "inner root" is not an example of a particular type of root, but of a peculiar habit which

exists in certain species of Lycopodium. The roots arise in acropetal succession at any point of the stele of the stem. Instead of penetrating the cortex at once and emerging as aerial roots, they turn down in

FIG 1.—Lycopodium pithyoides

the cortex, boring canals through long stretches of cortex, and emerge finally at or near the base. This habit is associated with erect forms of Lycopodium, both terrestrial and epiphytic. A similar habit is described in the Marattiaceae, SACHS (9) having given a brief description of its occurrence in *Angiopteris evecta*, in which the roots are said to bear a definite relation to the leaf bases.

The "inner roots" of Lycopodium were described by BRONGNIART (1), who stated that they were characteristic of erect dichotomous forms. STRASBURGER (11) described the more obvious features of the "inner roots" of *L. Selago*, and gave a list of about twenty species characterized by their presence. All the species noted are members of the Selago group except *L. Phlegmaria*. In none of the species described have the "inner roots" attained a development comparable to that in *L. pithyoides*. Few have been studied in sufficient detail to afford a basis for comparison. STRASBURGER records *L. Selago* as showing eight roots in a cross-section taken 5^{cm} above the base of the stem. BRONGNIART (2) figures *L. phlegmaria* and *L. verticillatum* with fifteen "inner roots." *Fig. 2* shows a cross-section of *L. pithyoides*, taken $10–12^{cm}$ above the base of the stem, in which fifty-two roots were found. The extraordinary development of these roots is no doubt an important factor in giving the stem its characteristic stoutness and rigidity.

The root primordia usually appear within $2–5^{mm}$ of the tip of the stem, but occasionally there will be none within $5–10^{cm}$ of the tip. The primordium shown in *fig. 3* and *fig. 4* was found about 2^{mm} from the apex of a stem which was about 25^{cm} high. The vascular cylinder of the stem was still in the promeristem stage.

The roots of *L. pithyoides* show four distinct initial regions for the plerome, periblem, dermatogen, and calyptrogen. The plerome arises from an initial region consisting probably of only one initial cell. It was impossible to determine with certainty the exact number of initials, but such preparations as that shown in *fig. 5* indicate a single initial; it is possible, however, that there are several, as in the forms studied by BRUCHMANN (3). The periblem initial region consists of several layers forming a well-defined although not sharply differentiated region. There is a well-defined one-layered dermatogen which gives rise to the epidermal cells and trichoblasts.

The trichomes arise from definite cells which are cut off by curved oblique walls from the original epidermal cells a short distance back of the apex of the root (*figs. 6, 7, 8*). With the growth of the epidermal cells and trichoblasts, the latter come to have the appearance of wedge-shaped cells. The outer wall rounds out and a papilla appears which rapidly elongates into a slender, rather thick-walled, persistent

trichome. The trichoblast usually divides once longitudinally, so that the trichomes are usually found in pairs (*fig. 9*). Since almost every epidermal cell gives rise to a trichoblast, the trichomes are abundant and very uniformly distributed. The trichoblastic cells are cut off as regularly in the "inner roots" as in the external, but of course produce trichomes only in aerial and soil roots. The formation of trichoblasts in Lycopodium was first described by NAEGELI and LEITGEB (**6**). LEAVITT (**5**) has described them in greater detail, giving *L. inundatum* as an example of the type in which the trichome is cut off by a straight anticlinal wall, and *L. lucidulum* as an example of the type with oblique walls. The development of the trichomes in *L. pithyoides* seems to be the same as that in *L. lucidulum*.

The root begins to grow downward immediately after leaving the stele (*fig. 10*), dissolving and crushing the cortex to make way for its rapid enlargement. The roots bend and curve, winding in and out around each other and around the leaf traces as they work their way down the stem. Several roots often run side by side for a considerable distance, so crowded together that the sheaths appear to fuse. PRITZEL (**7**) and STRASBURGER both describe branching as of frequent occurrence. It must be extremely rare in *L. pithyoides*, for no cross-sections have revealed the slightest trace of it, and an extensive dissection of the stem was equally unsuccessful. That it does occur is shown by a root-tip taken from an "inner root" which has the dichotomous forking so characteristic and abundant in external roots (*fig. 11*). It is possible that in such cases one branch may become abortive owing to the crowded condition under which the root is growing.

A typical "inner root" is shown in *fig. 12*. On the outside is a heavy sclerenchyma sheath, which in some roots attains a thickness two or three times as great. Within the sheath is a region of slightly thickened parenchyma which extends to the stele. The parenchyma region is the one which STRASBURGER erroneously called the outermost region of the root, because of the ease with which it is separated from the adjoining sclerenchyma. BRUCHMANN (**4**) in his discussion of *L. Selago* corrected this error. The tearing between the two regions is due not to a lack of organic connection, but to the weakness

of the middle lamella of the inner cells. It is so delicate that any pressure sufficient to section the sclerenchyma sheath is almost certain to cause a splitting of the middle lamella. Although the two regions are rather sharply defined, there are always a few cells in the transition stage. The thin-walled cortex is limited by the endodermis, which although not sharply differentiated can be easily recognized by its suberized walls.

In the genus Lycopodium there is great variation in the type of stele found in the root. Russow (**8**) has described that of *L. annotinum* and *L. complanatum* as being almost identical with that of the stem, but the stele of *L. Selago* and *L. inundatum* as being strikingly different. *L. pithyoides* is similar to *L. Selago*. The vascular strand is collateral, with a crescent-shaped xylem, the phloem lying between the horns of the crescent. The protoxylem is found regularly occupying the horns, but occasionally it spreads along the outer region of the metaxylem. There is no evident differentiation into protophloem and metaphloem either in root or stem. Russow in his discussion of *L. Selago* regards the parenchyma region outside the xylem as belonging to the phloem, and consequently classes the bundle as concentric. This indefinite region may be one or two cells thick or may be altogether lacking, and it seems better to regard it as pericycle rather than as phloem. It consists of small thin-walled parenchyma cells containing protoplasm and nuclei, with none of the wide-mouthed thick-walled cells which characterize the phloem region. The shape of the xylem strand varies somewhat in different parts of the root. The origin from the stele is seen in *figs. 13* and *14*. It is connected with two of the xylem strands and one of the phloem; this arrangement is shown in the primordium stage shown in *fig. 4*. As the root leaves the stele of the stem, the xylem strands move together, forming a U-shaped figure, with the closed end toward the center of the stem (*figs. 15* and *16*.) As the root passes down the stem, the xylem becomes massed together, so that when the root leaves the stem it has the form shown in *fig. 17*, which is the arrangement characteristic of external roots. The behavior of the xylem during forking of the root is shown in *figs. 18, 19, 20*. The xylem divides at the place where the two strands had previously united, and each strand rapidly assumes the characteristic crescent-shaped form.

The region of exit of the roots extends from the base of the stem to about 1 cm above. BRUCHMANN (3) found that in *L. Selago*, when the stem was closely appressed to the ground, an earlier exit was favored. The root may bend abruptly and leave the stem at a right angle, or it may run around the stem for a short distance not far below the epidermis, leaving the stem at a tangent; but it is more usual for the root to turn gradually toward the outside of the stem and leave it obliquely. The root hairs which in the "inner roots" develop no further than the trichoblast stage, develop extensively and form a dense mat around the external root (*fig. 21*). The epidermis, which soon disappears in the "inner root," is persistent in the external. In an old stem in which the departing roots are numerous and thickly crowded, the cortical tissue of the stem becomes torn and fragmentary. Most of it disappears, leaving a brush-like mass of roots with very little cortex between them.

This investigation was conducted at the University of Chicago under the direction of Professor JOHN M. COULTER and Dr. W. J. G. LAND, and arose incidentally in connection with an investigation of the development of the stele of Lycopodium.

THE UNIVERSITY OF CHICAGO

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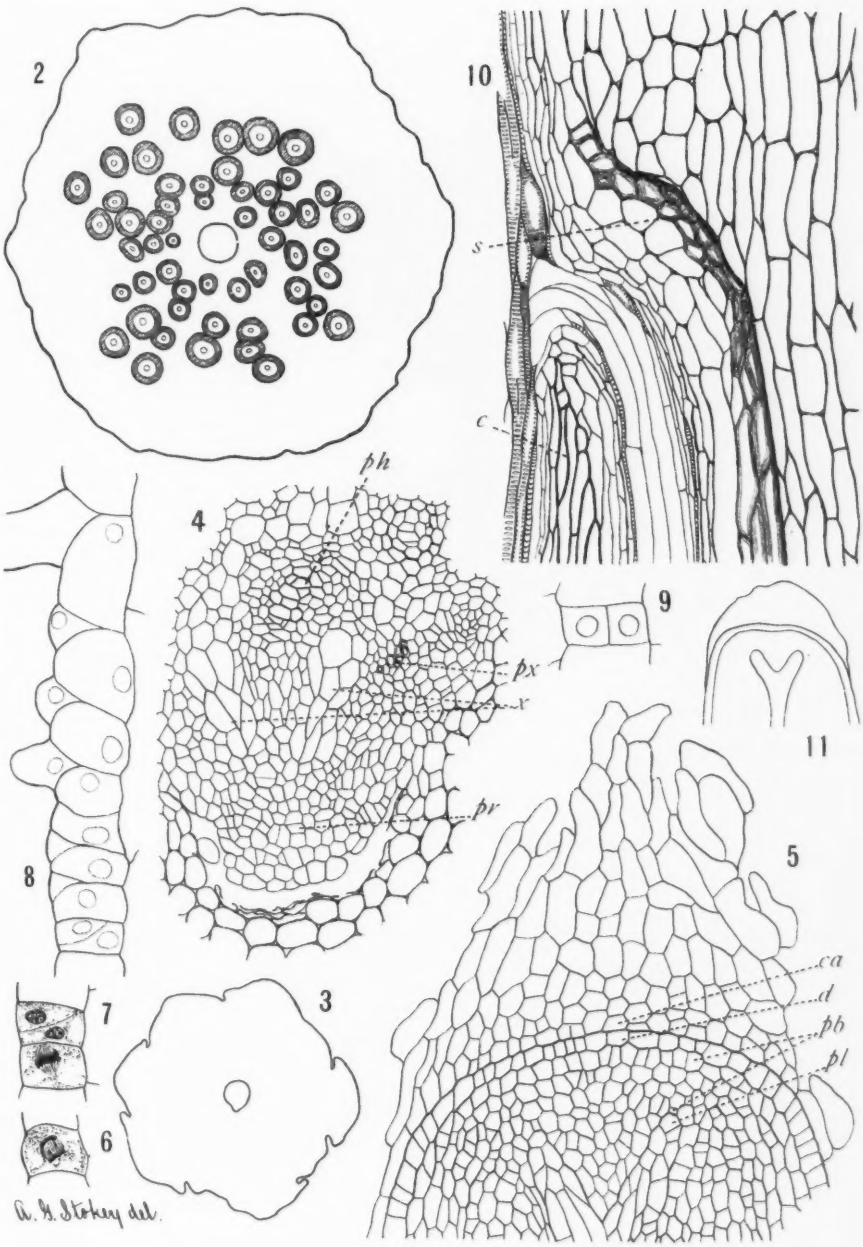
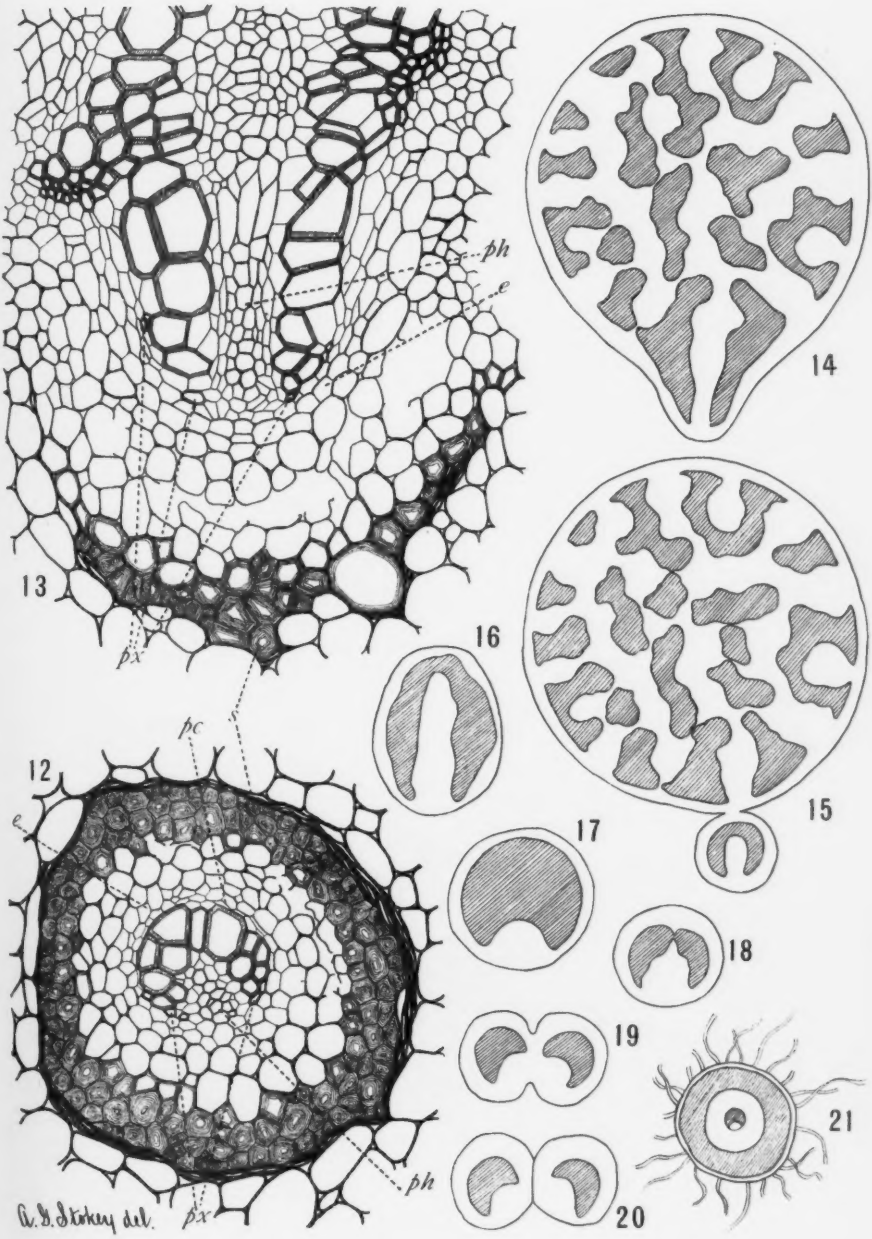


STOKEY on LYCOPODIUM



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EXPLANATION OF PLATES V AND VI

All the figures were drawn with the aid of an Abbé camera lucida and reduced one-half in reproduction. Abbreviations are as follows: *x*, xylem; *px*, protoxylem; *ph*, phloem; *e*, endodermis; *pc*, pericycle; *c*, cortex; *sc*, sclerenchyma; *pl*, plerome; *pb*, periblem; *d*, dermatogen; *ca*, calyptrogen; *pr*, primordium.

PLATE V

FIG. 2. Cross-section of stem showing stele surrounded by "inner roots." ×8.

FIG. 3. Cross-section of stem with primordium of root. ×8.

FIG. 4. Primordium of root at its origin from stele of stem. ×160.

FIG. 5. Longitudinal section of root tip. ×160.

FIGS. 6–8. Dermatogen of root showing development of trichomes. ×375.

FIG. 9. Surface view of trichoblast cell which has divided to form two trichomes. ×375.

FIG. 10. Longitudinal section through "inner root" at origin. ×80.

FIG. 11. Diagram of a root-tip of an "inner root," showing dichotomy.

PLATE VI

FIG. 12. Cross-section of "inner root." ×180.

FIG. 13. Cross-section of "inner root" at origin. ×180.

FIG. 14. Cross-section of stele of stem at origin of "inner root" (xylem strands shaded). ×60.

FIG. 15. Cross-section of stele of stem showing root almost separated from stele. ×60.

FIG. 16. Cross-section of stele of root showing shape of xylem just after the root leaves the stele of the stem. ×180.

FIG. 17. Cross-section of stele of root just above the place where the root leaves the stem. ×180.

FIGS. 18–20. Cross-section of stele of root showing division of xylem during forking of the root. ×180.

FIG. 21. Cross-section of external root. ×25.

BRIEFER ARTICLES

THE BLACK-FRUITED CRATAEGUS OF WESTERN NORTH AMERICA

Mr. W. N. SUKSDORF has sent me an interesting series of specimens of black-fruited Crataegus collected by him in the neighborhood of Bingen, Klickitat County, western Washington, and has called my attention to some distinct variations in different individuals of these plants. He groups his plants as follows:

1 (*D*). Plants with normally twenty stamens; fruit usually in few-fruited clusters, generally not more than 8^{mm} in diameter, and ripening sometimes as early as the first of July and sometimes not until the middle of August.

2 (*X*). Plants with rather larger flowers; normally ten stamens; fruit in large drooping clusters often 1 to 1.2^{cm} in diameter and ripening in July. This form seems to be the *C. Douglasii* Lindley of English gardens.

3 (*Y*). Plants with normally ten stamens; fruit in few-fruited often erect clusters, usually 8 to 10^{mm} in diameter, and ripening from July to September. This and the following I cannot distinguish from the *X* group.

4 (*Z*). Plants with ten stamens; fruit as small as that of *D* and ripening sometimes as late as September.

The black-fruited thorn of the northwest was discovered by DAVID DOUGLAS, and in 1830 was named by LINDLEY *C. Douglasii* (*Bot. Reg. pl. 810*). The figure in the *Botanical Register* represents the flowers with ten stamens, and in a grafted plant imported from Europe, which I have cultivated for more than thirty years and which I believe to be one of the same strain as the plant formerly in the garden of the Royal Horticultural Society at Chiswick from which LINDLEY'S figure and description were made, the stamens are normally ten, although sometimes by abortion they are as few as five. The stamens of a tree of *C. Douglasii* sent to the Arnold Arboretum many years ago by MAX LEICHTLIN of Baden Baden are mostly twenty, but are sometimes reduced to twelve or fifteen. The two trees in other characters appear identical, and except in the number of the stamens I have been unable to find any character by which these two forms can be safely distinguished.

On all the specimens of this black-fruited form which I have seen the leaves vary from obovate to ovate; they are cuneate at the base, rather

coarsely serrate occasionally nearly to the base, and lobed only toward the apex. At the flowering time they are lustrous, covered above with soft white hairs most abundant on the midribs and veins, and pale and more or less villose in the axils of the veins below, later becoming subcoriaceous, dark green, and very lustrous. The flowers vary from 1.2 to 1.4^{cm} in diameter and are produced on long slender glabrous pedicels, in usually 15- or 16-flowered compound corymbs, with linear caducous bracts and bractlets. The broad calyx-tube is quite glabrous and much longer than the wide, entire, or occasionally minutely dentate lobes, acute and bright red at the apex and sparingly villose on the inner surface. The anthers are small and very pale rose color, and the styles are normally five. The fruit is black, short-oblong, crowned by the persistent calyx, with yellow, succulent, rather sweetish flesh; the nutlets, which in many of Mr. SUKSDORF'S specimens are abortive, wrinkled, and much reduced in size, are normally five, obtuse at the ends, slightly ridged on the back, and irregularly penetrated on the inner faces by shallow longitudinal cavities. The branchlets are red or orange-red and very lustrous, and the spines, which are stout and nearly straight, and generally not more than from 1 to 1.5^{cm} in length, occasionally on vigorous branches become nearly 4^{cm} long. *C. Douglasii* is the common species of the northwestern states, extending north into British Columbia, south into northern California, and eastward at least as far as Wyoming.[1]

Of the plants collected by Mr. SUKSDORF, those with twenty stamens and small fruit, placed by him in his group *D*, vary most from what I consider the type of *C. Douglasii*, and although it does not seem possible to distinguish these specifically, they certainly constitute a well-marked variety, for which I suggest the name:

CRATAEGUS DOUGLASII var. **Suksdorfii,** nov. var.—A shrub sometimes 8^{m} high, with numerous stout, erect, and spreading stems.—Banks of the Columbia River and borders of bottom lands, West Klickitat County, Washington, *W. N. Suksdorf*, 1905–1906 (nos. 4034, 4919, 5026, 5031, 5040).

Another plant in this group should also be mentioned. This is a ten-stamened plant with distinctly chestnut-colored fruits, and may be called:

CRATAEGUS DOUGLASII forma **badia,** nov. forma.—It grows on Union Flat, six miles south of Pullman, Washington, with the black-fruited *C.*

[1] Of the black-fruited Crataegus which grows at a few stations on the northern peninsula of Michigan and on some of the islands of Lake Superior, and which has been referred to *C. Douglasii*, flowers have not been collected and its true specific position cannot be determined at present.

Douglasii which, although it flowers ten days later, it otherwise resembles except in the color of the fruit. This interesting plant has been collected at different times between 1896 and 1902 by *Mr. C. V. Piper* (nos. 2358, 2454, 3599, 3810, and 3826).[2]

The following synopsis of the species of the DOUGLASAE group may aid in the determination of the species:

Anthers slightly tinged with rose color; calyx-lobes entire or minutely dentate, sparingly villose on the inner surface; fruit short-oblong to subglobose; nutlets 5, obtuse at the ends, slightly ridged on the back, irregularly penetrated on the inner face by shallow longitudinal grooves.

Leaves subcoriaceous, very lustrous, obovate to broadly ovate, coarsely serrate, usually lobed toward the apex, more or less villose while young, generally becoming glabrous; stamens 5–20, normally 10; spines numerous, usually short and stout. . . . . 1. *C. Douglasii.*

Leaves thinner, lanceolate to oblong-obovate, acute at the ends, finely serrate, not lobed, covered above while young with soft white hairs, soon glabrous; stamens 10; spines few, long and slender or wanting . . . 2. *C. rivularis.*[3]

—C. S. SARGENT, *Arnold Arboretum.*

[2] *C. Gaylussacia* Heller (Bull. South. Cal. Acad. Sci. **2**:68. 1903) is one of the black-fruited group and was collected by HELLER, August 20, 1902, at Sebastopol in Sonoma County, California. Flowers have not been seen, and I suspect that it will turn out to be one of the small-fruited forms of *C. Douglasii.* The leaves are smaller, however, and rather thinner than those of the ordinary forms of that species, and they are still slightly hairy on the upper surface at the end of August when the fruit appears to have been fully ripe.

[3] *C. rivularis* is a more southern species than *C. Douglasii* and is confined to the interior of the continent. It is most abundant on the Wasatch Mountains of Utah and ranges to southwestern Colorado and western Wyoming.

CURRENT LITERATURE

MINOR NOTICES

Mechanics of cell division.—In a study of the mechanics of cell division, GIESENHAGEN[1] presents the following conclusions. The nucleus, even in the resting condition, has a definite polarity, so that it can divide only in a predetermined direction. The median plane in which the cell plate will appear at mitosis is the equatorial plane, and the axis is perpendicular to its center. The axis of the nucleus is definitely determined by the preceding mitosis, its most common position being that of the axis of the mother cell, or a position at right angles to the axis of the mother cell. If the nuclei maintain their positions without any movement, there will be a succession of anticlines and periclines, but many causes may change the position of the nucleus and affect the direction of its axis and consequently the plane of the wall to be formed when the nucleus divides. The numerous variations from the periclinal and anticlinal divisions are discussed and suggestions are made as to their causes.—CHARLES J. CHAMBERLAIN.

American Breeders' Association.—The proceedings of the meeting held at Lincoln, Nebraska, January 17–19, 1906, have just been published as the second volume issued by the Association. Naturally the papers vary widely in subject and in value, and it is impossible to estimate them by reading. It is clear, however, that fundamental biological problems are being considered; and that views and data are being recorded that are extremely suggestive. Among the strictly botanical topics may be noted the following: Tobacco breeding, The corn plant as affected by date of planting, Some correlated characters in wheat and their transmission, Plant adaptation, A method of breeding a strain of alfalfa from a single individual, Correlation of characters in plant breeding, Variation in wheat hybrids, Breeding drought-resistant crops, Fundamental requirements for grain breeding, Value of corn pollen from suckers *vs.* main stalks, A theory of heredity, etc.—J. M. C.

The Dillenian Herbaria.—The herbarium of the University of Oxford contains some collections of great historical interest, among which those of DILLENIUS (1684–1747), the first "Sherardian" professor of botany, are conspicuous. An account of the Dillenian herbaria has just been published, together with a biographical sketch, selections from correspondence, etc., by DRUCE,[2] edited and with an introduction by VINES, the present "Sherardian" professor. The collec-

[1] GIESENHAGEN, K., Studien über die Zellteilung im Pflanzenreiche, ein Beitrag zur Entwickelungsmechanik vegetabilischer Gewebe. 8vo. pp. 91. Stuttgart: Fr. Grub. 1905.

[2] DRUCE, G. CLARIDGE, and S. H. VINES, The Dillenian herbaria. pp. cxii + 258. Oxford: The Clarendon Press. 1907. 12 *sh.*

tions of DILLENIUS relate to "subjects so diverse as the investigation of the British Flora, especially that of Wales; the botanical exploration of North America; the botany of the exotics, especially succulents, cultivated in his time; and the classification of cryptogamic plants." The Dillenian herbaria are three: that of the *Synopsis;* that of the *Hortus Elthamensis;* and that of the *Historia Muscorum.*—J. M. C.

Plant chemistry.—The original papers and addresses on plant chemistry, published by HELEN C. DeS. ABBOTT MICHAEL (*b.* 1857, *d.* 1904), have been collected and reprinted, together with a biographical sketch (110 pp.) and four literary papers, to form a neat volume.[3] Mrs. MICHAEL'S scientific training was obtained under extraordinary difficulties, and her work was made somewhat scattering by reason of the very alertness and eagerness of her mind. Her contributions to a knowledge of the constituents of plants, especially the glucosides, are more valuable than her theories as to the correlation of plant form and classification with the nature and distribution of chemical compounds. The book in its editing shows some unfamiliarity with scientific names and terms. It is a worthy memorial to one whose work was cut short by an untimely death.—C.R.B.

Flora of Guatemala.—JOHN DONNELL SMITH has published as the eighth part of his *Enumeratio* an index[4] of the preceding parts. The species are arranged alphabetically, and 7979 numbers are cited, representing 3736 species, 1189 of which are not contained in HEMSLEY'S *Biologia Centrali-Americana.* Incorrect determinations and faulty nomenclature are also corrected. The author is to be congratulated on the complete way in which he has investigated the flora of this region and organized his results.—J. M. C.

London botanic gardens.—M. PERRÉDÈS has brought together in a single publication[5] a series of papers that appeared in the *American Journal of Pharmacy.* The three gardens described are Kew Gardens, The Royal Botanic Society's Gardens in Regent's Park, and The Chelsea Physic Garden. The numerous admirable illustrations that accompany the text make these gardens very real to the reader.—J. M. C.

Pflanzenfamilien.[6]—Parts 227 and 228 contain the completion of the Lembophyllaceae, and the presentation of Entodontaceae, Fabroniaceae, Pilotrichaceae, Nematoceae, and Hookeriaceae, by V. F. BROTHERUS. The third part of the

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[3] MICHAEL, H. A., Studies in plant and organic chemistry. 8vo. pp. 423. Cambridge: The Riverside Press. 1907. $2.50.

[4] SMITH, JOHN DONNELL, Enumeratio plantarum Guatemalensium etc. Pars VIII. pp. 221. Oquawka, Illinois: H. N. Patterson. 1907.

[5] PERRÉDÈS, PIERRE ÉLIE FÉLIX, London Botanic Gardens. pp. 100. *pls. 31.* London: The Wellcome Chemical Research Laboratories. 1907.

[6] ENGLER, A., und PRANTL, K., Die natürlichen Pflanzenfamilien. Lief. 227 und 228 und Ergänzungsheft II, Lief. 3. Leipzig: Wilhelm Engelmann. 1907.

second supplement continues the literature of 1899–1904 in reference to dicotyledons from Euphorbiaceae to Sapotaceae.—J. M. C.

Flora of Winneshiek Co., Iowa.—SHIMEK[7] has published an account of the plants of one of the counties of Iowa, prefacing the annotated list by a discussion of the forest problem, ornamental plants, forage plants, weeds, and medicinal plants.—J. M. C.

Eucalyptus.—The eighth part of MAIDEN'S[8] revision of Eucalyptus contains the description, synonymy, range, and affinities of seven species. This series, begun in 1903, now includes twenty-four species.—J. M. C.

Das Pflanzenreich.[9]—Part 28 contains the group Calceolarieae of Scrophulariaceae. The three genera are Porodittia (1 sp.), Jovellana (6 spp.), and Calceolaria (192 spp., with 9 new).—J. M. C.

NOTES FOR STUDENTS

Apogamy and apospory in ferns.—Professor FARMER and Miss DIGBY have published[10] the results of their studies of apogamy and apospory in ferns. The forms described are *Lastrea pseudo-mas* vars. *polydactyla* Wills, *polydactyla* Dadds, and *cristata apospora* Druery; *Athyrium Filix-foemina* vars. *clarissima* Jones, *clarissima* Bolton, and *uncoglomeratum* Stansfield; and *Scolopendrium vulgare* var. *crispum Drummondae.*

In *Athyrium Filix-foemina clarissima* Jones there is no change in the number of chromosomes in passing from the sporophyte to the gametophyte phase of the life-history; and there is no migration of nuclei from one prothallial cell to another. The embryo arises as a bud upon the gametophyte.

In *Athyrium Filix-foemina clarissima* Bolton there is no reduction of chromosomes, no true fertilization, no migration of prothallial nuclei, and the embryo develops from an unfertilized egg.

In *Athyrium Filix-foemina uncoglomeratum* Stansfield the embryo arises in connection with an archegonium, but details were not discovered. The number of chromosomes (about 100) is maintained throughout the life-history and there is no migration of prothallial nuclei.

In *Scolopendrium vulgare crispum Drummondae* a remarkable condition is described. The number of chromosomes in sporophyte nuclei is about 100, in prothallial nuclei about 70, in archegonial nuclei about 80, and in antheridial

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[7] SHIMEK, B., Flora of Winneshiek County. Iowa Geol. Surv. **16**:147–211. 1906.

[8] MAIDEN, J. H., A critical revision of the genus Eucalyptus. Part VIII. pp. 211–254. *pls. 37–40.* Sydney: Published by State of New South Wales. 1907. *2s. 6d.*

[9] ENGLER, A., Das Pflanzenreich. Heft 28. Scrophulariaceae Antirrhinoideae-Calceolarieae von Fr. Kränzlin. pp. 128. *figs. 21 (142).* Leipzig: Wilhelm Engelmann. 1907. *M*6.40.

[10] FARMER, J. BRETLAND, and L. DIGBY, Studies in apogamy and apospory in ferns. Annals of Botany **21**:161–199. *pls. 16–20.* 1907.

nuclei between 70 and 82. There is no cyclic alteration in the number of chromosomes, and no migration or fusion of nuclei of prothallial cells. The embryo arises from the unfertilized egg.

In *Lastrea pseudo-mas polydactyla* Wills, the authors still maintain their claim, made in a preliminary paper, that there is a migration and fusion of prothallial nuclei, thus initiating the sporophytic phase. There is a normal reduction of chromosomes.

In *Lastrea pseudo-mas polydactyla* Dadds there is a reduction of chromosomes and the sporophyte is initiated by a migration and fusion of prothallial nuclei. The embryo, however, comes from a projection which may be regarded as an arrested archegonium.

In *Lastrea pseudo-mas cristata apospora* Druery there is no reduction of chromosomes and no migration of prothallial nuclei. The number of chromosomes shows a remarkable variation, there being 60 in nuclei of prothallial cells, about 78 in nuclei of the embryo, and about 90 in nuclei of antherozoids.

These various forms may be arranged in two categories, one in which spores are produced in connection with the usual reduction of chromosomes; and the other in which there is no spore formation or reduction of chromosomes, the embryo appearing as an outgrowth from the sporophyte. The wide variation in the number of chromosomes is not thought to be due to inaccuracy in counting, but to a real difference. The authors believe that the fluctuation might seem to negative any value being attached to the number of chromosomes.

After a lengthy discussion a general conclusion is drawn which is quite at variance with current notions, namely that there is no necessary correlation between the periodic reduction in the number of chromosomes and alternation of generations. Fertilization and reduction, however, are recognized as holding a definite causal relation to each other, but without assuming any necessary connection between either of them and any other features in the life-history.—CHARLES J. CHAMBERLAIN.

Apogamy in Marsilia.—While investigating apogamy in Alchemilla, STRASBURGER[11] realized the desirability of examining apogamy in other groups. Since Marsilia had been reported to produce embryos without fertilization, he secured material from various botanists, and even obtained sporocarps from specimens in the Kew and Berlin herbaria. Some of the sporocarps, known to have been collected more than thirty years ago, germinated readily.

In some species, notably *M. Drummondii*, the megaspores when isolated from microspores produce prothallia and embryos almost as abundantly as when microspores are present. Sections show that embryos often develop from eggs without fertilization, and that nuclei of these embryos have 32 chromosomes, the $2x$, diploid, or sporophyte number, as was found by comparing mitoses in root tips and other vegetative structures. Megaspores are formed which have $2x$ chromosomes in their nuclei, the reduction of chromosomes having failed to take

[11] STRASBURGER, E., Apogamy bei Marsilia. Flora **97**:123–191. *pls. 3–8.* 1907.

place. Such megaspores form prothallia whose nuclei have $2x$ chromosomes, and finally an egg is formed with $2x$ chromosomes, and this egg develops an embryo with $2x$ chromosomes; so that the sporophyte number of chromosomes is maintained throughout the life-history. But in the same species there are instances of reduction of chromosomes, so that prothallia and egg have the x or gametophyte number.

In forms which produce apogamous embryos, microsporogenesis is likely to be abnormal, the development often stopping before the nucleus of the microspore mother cell divides; but here again normal microspores with the reduced number of chromosomes are sometimes formed. The nuclei of the x and $2x$ prothallia can be distinguished in a general way by their size, the diameter of the nuclei in $2x$ prothallia being about one-third greater than those in x prothallia; also the diameter of the eggs with $2x$ chromosomes is about one-fourth greater than that of eggs with the reduced number.

STRASBURGER uses the term apogamy rather than parthenogenesis because he regards an egg with $2x$ chromosomes as a purely vegetative cell. He would use the term parthenogenesis only in case an egg with x chromosomes should develop an embyro without fertilization. Even if $2x$ chromosomes should appear at the first mitosis in such an egg, he would still regard it as a genuine case of parthenogenesis.

The preparations naturally showed many stages in the development of spore membranes. Shortly before the spore reaches the periphery of the vesicle containing it, the perinium appears. This is a delicate, fine-pored membrane (*Häutchen*) laid down upon the surface of the vesicle by the surrounding tapetal plasmodium. The prismatic layer is then laid down upon the delicate membrane. The exine, mesopore, and endospore are then developed in succession upon the protoplast of the spore. This study supports the view that cell membranes arise only in direct relation with protoplasm.—CHARLES J. CHAMBERLAIN.

Items of taxonomic interest.—S. M. BAIN and S. H. ESSARY (Jour. Mycol. 12:192, 193. 1906) have described a new anthracnose of alfalfa and red clover (*Colletotrichum trifolii*), which is said to be the most serious plant disease occurring in Tennessee.—J. M. GREENMAN (Field Columbian Mus. Publ. Bot. Ser. 2:185–190. 1907) has published 10 new species of Citharexylum as preliminary to a synoptical revision of the genus.—E. HASSLER (Bull. Herb. Boiss. II. 7:161–164. *figs.* 5. 1907) has described a new genus (*Dolichopsis*) of Leguminosae from Paraguay.—L. A. DODE (*idem* 247, 248. *figs.* 3) has described a new species of Juglans (*J. elaeopyren*) from the Santa Catalina Mountains of Arizona; it is distributed in Pringle of 1881 as *J. rupestris* Engelm., and the type is in Herb. Barbey-Boissier.—A. H. MOORE (Proc. Amer. Acad. 42:521–569. 1907), in a revision of the confused genus Spilanthes, recognizes 63 species; describes as new 2 subsections, 8 species, 4 varieties, and 7 forms; and makes 14 new combinations.—W. H. BLANCHARD (Torreya 7:55–57. 1907) has described a new Rubus (blackberry) from the vicinity of Philadelphia and Washington.—J. R.

DRUMMOND (Kew Bull. 1907:90–92) has described a new genus (*Chlamydites*) of Compositae from Tibet.—H. D. HOUSE (Muhlenbergia 3:37–46. 1907) has described 15 new species of Ipomoea from Mexico and Central America.—REHDER (Rhodora 9:60–62. 1907) has published a new variety of *Quercus prinoides* from Mass. and N. J.—FINET and GAGNEPAIN (Bull. Soc. Bot. France IV. 6:55–170. *pls. 9–20.* 1906), in continuation of their *Flore de l'Asie orientale*, have presented the Anonaceae, including 25 genera represented by 249 species, 28 of which are new.—O. E. JENNINGS (Ann. Carnegie Mus. 4:73–77. *pl. 20.* 1906) has described a new species of Lonicera (*L. altissima*) from Penn.—H. D. HOUSE (Bull. Torr. Bot. Club 34:143–155. 1907), in his third paper on N. Am. Convolvulaceae, has described a new species of Calycobolus.—K. K. MACKENZIE (*idem* 151–155) has described 4 new western species of Carex.—W. W. ROWLEE (*idem* 157–159) has described 2 new species of Salix from the Canadian Rocky Mountains.—J. M. C.

Riella and Sphaerocarpus.—In the eleventh of his *Archegoniatenstudien* GOEBEL discusses germination and regeneration in these aberrant liverworts,[12] in extension of the investigations recorded in no. IV. Having an abundance of living material, Riella being easily cultivated, he is able to show that the peculiar "wing" of Riella is not an "outgrowth" from an earlier-formed midrib, but that the germ-disk lies in the same plane as the wing and expands directly into the plant (homoblastic development), as soon as the intercalary growing point is organized and becomes active.

Herein he controverts SOLMS-LAUBACH and PORSILD, who seem to consider the development as heteroblastic, holding that the thallus arises, as a different structure, so to speak, from the germ-disk (which SOLMS called protonema and PORSILD the primordial lobe), much as a sphagnum stem arises from its prothallus. GOEBEL (who, however, denies any special interest in such a question), in agreement with PORSILD but contrary to SOLMS, finds no wedge-shaped apical cell in the growing point until a late stage of development. When after injury or under bad conditions adventive shoots are formed, whether on the thallus or germ-disk, there is first the production of simple one-layered regions like the germ-disks, i.e., secondary disks, and from these the new thalli arise directly, as in sporelings.

In Sphaerocarpus the spore produces a germ tube, which at its end becomes a multicellular cylinder with a depressed tip. From one quadrant of the cupped end the vegetative point arises and from two others the wings proceed. The germ cylinder thus produces the thallus homoblastically. In adventive shoots the same phenomena recur. GOEBEL confirms LEITGEB'S statement as to the precocious formation of sex organs, plants only 0.1^{mm} showing their rudiments.[13] The largest sterile thallus found (and this was far beyond the usual) was 1^{mm} long.—C. R. B.

[12] GOEBEL, K., Archegoniatenstudien. XI. Weitere Untersuchungen über Keimung und Regeneration bei Riella und Sphaerocarpus. Flora 97:192–214. *figs. 23.* 1907.

[13] Misquoted by CAMPBELL, Mosses and Ferns, 2 ed., 82, as "one millimeter."

Respiration.—In accordance with the theory that in aerobic respiration oxygen acts as a depolarizer,[14] PACKARD finds[15] that when carbohydrates which can be absorbed (e. g., maltose, glucose, fructose) are injected peritoneally into *Fundulus heteroclitus* the resistance to deprivation of oxygen is greatly increased; which the author explains on the assumption that the sugars act as depolarizers. He also concludes that the decrease in resistance to lack of oxygen shown by Fundulus embryos in successive stages of development is due to the using-up of material (probably carbohydrates) stored in the egg.

A paper has been published by HRUBY,[16] which conforms in general to the more modern views as to the nature of respiration; and, though it adds nothing new, it will aid in the propagation of better ideas among European students.

KOSTYTSCHEW reports that *Aspergillus niger* fed with sugar produces little CO_2 under anaerobic conditions in a gaseous medium, but when submerged in a sugar solution it produces CO_2 and alcohol abundantly in the same ratio as in alcoholic fermentation.[17]

PALLADIN and KOSTYTSCHEW concluded that the anaerobic respiration of frozen lupines and stems had nothing to do with alcoholic fermentation.[18] Now they add[19] that alcohol formation (in consequence of anaerobic respiration of seed-plants) only occurs in the presence of carbohydrates; and when they are absent CO_2 is formed without alcohol. The chemics of this sort of anaerobic respiration they leave for further investigation.—C. R. B.

The algal flora of the tropics.—FRITSCH[20] has reached the following conclusions from a study of the subaerial and freshwater algae of the tropics. There is evidence that in the damp tropics there is always a very extensive subaerial algal covering, which probably consists almost entirely of Cyanophyceae, which may thus be regarded as an essentially tropical group. This group may be the descendants of primitive algal forms which existed at earlier periods under conditions analogous to those found in the damp tropics at the present day. Trentepohlia is the only genus of green algae that is really successful in the subaerial flora. In

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[14] BARNES, C. R., The theory of respiration. BOT. GAZETTE **39**:96. 1905. MATHEWS, A. P., A theory of the nature of protoplasmic respiration and growth. Biol. Bull. **8**:331. 1905.

[15] PACKARD, W. H., The effect of carbohydrates on resistance to lack of oxygen. Am. Jour. Physiol. **18**:164–180. 1907.

[16] HRUBY, J., Die Atmung der Pflanzen. Beih. Bot. Centralbl. **21**[1]:156–172. *figs.* 3. 1907.

[17] KOSTYTSCHEW, S., Ueber die Alkoholgärung von *Aspergillus niger*. Ber. Deutsch. Bot. Gesells. **25**:44–50. 1907.

[18] BOT. GAZETTE **42**:397. 1906.

[19] PALLADIN, W., and KOSTYTSCHEW, S., Ueber anaerobe Atmung der Samenpflanzen. Ber. Deutsch. Bot. Gesells. **25**:51–56. 1907.

[20] FRITSCH, F. E., The subaerial and freshwater algal flora of the tropics; a phytogeographical and ecological study. Annals of Botany **21**:235–275. 1907.

the submerged algal flora of tropical freshwaters the Cyanophyceae also constitute a very important element, though not as preponderant as in the subaerial flora. This freshwater flora is composed of forms in which narrow filaments are much more abundant than broad ones, a fact thought to be related to the small amount of dissolved oxygen in the water. Cladophora and Rhizoclonium are very poorly represented, and Vaucheria and Botrydium are very rare. The Confervales are not very well represented, and probably the Ulotrichales do not attain much development. Spirogyra, on the other hand, is even more abundant in the tropics than in northern latitudes. The desmids show a marked filamentous tendency, which may be due to poor aeration. Oedogonium is very abundant, and the freshwater red algae are not at all uncommon.—J. M. C.

Gametophytes and embryo of Libocedrus.—LAWSON[21] has added Libocedrus to the investigated Cupressineae and shows that it agrees in all essential characters with the other genera of the group, as may be seen from the following outline of the results. No prothallial cells are formed in the pollen grain, which at shedding contains the generative and tube nuclei. The pollen tube advances very directly to the archegonial chamber, and upon its arrival the body cell divides to form two large and equal male cells. One to three megaspore mother cells occur and each gives rise to a tetrad. The functioning megaspore becomes filled with endosperm tissue in the usual way, and the megaspore membrane is poorly developed. The archegonia range in number from six to twenty-four, and are grouped in a single complex, invested by a single layer of jacket cells. The ventral canal cell, as in other Cupressineae, is represented only by its nucleus. The contents of the pollen tubes are discharged into the common archegonial chamber, so that both male cells in a tube may function. In fertilization the male slips from its cytoplasmic sheath and unites with the egg nucleus. The two nuclei arising from the division of the fusion nucleus pass to the bottom of the egg and by two successive divisions form eight free nuclei before wall-formation. The cells of the proembryo are arranged in the usual three tiers.—J. M. C.

Evolution of the vascular system of ferns.—TANSLEY[22] has begun the publication of a series of lectures on the evolution of the vascular system of the Filicineae, the first considering the origin of the pteridophytes. The lecturer commits himself to the following positions: that bryophytes and pteridophytes have arisen from the algae independently; that the alternation of generations of the former is "antithetic," of the latter "homologous;" that the prevailing habit of dichotomy among the Filicineae indicates ancestors of the dichotomously branching thallus type, in which the "sporangiferous thallus became specialized for assimilatory functions;" that from such forms the "Filicales, Sphenophyllales, and Equise-

[21] LAWSON, ANSTRUTHER A., The gametophytes and embryo of the Cupressineae with special reference to *Libocedrus decurrens*. Annals of Botany **21**:281–301. *pls. 24–26.* 1907.

[22] TANSLEY, A. G., Lectures on the evolution of the filicinean vascular system. I. New Phytol. **6**:25–35. 1907.

tales originated, the first group retaining the characters of the primitive type;" and that the Lycopodiales "may perhaps be regarded as an extreme case of leaf reduction in one of these lines."—J. M. C.

Apogamy in Hieracium.—ROSENBERG[23] finds that in *Hieracium auricula* and *H. venosum* the development of pollen and embryo sacs is normal and fertilization occurs regularly; there are 9 chromosomes in the gametophyte, 18 in the sporophyte, and 27 in the endosperm. Most species of Hieracium, however, are apogamous, and their embryo sacs are formed without any reduction division. Usually they do not arise from megaspores, but from cells of the integument or chalaza; that is, the gametophyte is aposporous and contains the sporophytic number of chromosomes. In *H. excellens* an embryo sac with the reduced number of chromosomes is often formed, but there are usually aposporous sacs in the same head. This shows why *H. excellens*, in spite of its being apogamous, may also produce hybrids.—CHARLES J. CHAMBERLAIN.

Geographic distribution of closely related species.—LEAVITT[24] has published a study of the distribution of species in reference to their evolution. The topics discussed are "the effects of different evolutionary agencies upon specific distribution," "the necessity of isolation and Mendelian inheritance," "specific distribution in the animal kingdom," "the distribution of plants," and "evidence from North American Orchidaceae." The problems are stated and solutions are not attempted, but the impression of the author is that a study of the specific distribution of plants is not likely to be unfavorable to mutation as one method of evolution. He thinks that "the adherents of mutation will be able to bring forward enough cases of social distribution to render phytogeographic weapons useless in the attack upon this theory."—J. M. C.

Cytology of aposporv.—*Nephrodium pseudo-mas* Rich. var. *cristata apospora* Druery, according to Miss DIGBY,[25] shows almost all grades of apospory and apogamy except parthenogenesis. Several fronds pegged down quickly produced prothallia at the tips of the leaves, and these prothallia within three weeks bear apogamous embryos. Antheridia are not uncommon, but no archegonia could be found. The number of chromosomes in cells of the leaf, aposporous prothallia, and in the apogamous embryos is about 50, there being no reduction of chromosomes. In *N. pseudo-mas* Rich. var. *polydactyla* Wills, migrating nuclei, some of which have been seen to fuse, are a characteristic feature; while in the var. *cristata apospora* Druery there seems to be no migration or fusion of nuclei. —CHARLES J. CHAMBERLAIN.

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[23] ROSENBERG, O., Cytological studies on the apogamy in Hieracium. Bot. Tidsskrift **28**:143–170. *pls. 1–2.* 1907.

[24] LEAVITT, ROBERT GREENLEAF, The geographic distribution of closely related species. Amer. Nat. **41**:207–240. 1907.

[25] DIGBY, L., On the cytology of apogamy and apospory. II. Preliminary note on apospory. Proc. Roy. Soc. London **76**:463–467. 1905.

Myxomycete studies.—Degenerating nuclei have been observed in young sporangia of various Myxomycetes. JAHN[26] finds that at an early stage in the development of the sporangium nuclei fuse in pairs, and that the degenerating nuclei are those which have failed to fuse. Preceding sporogenesis JAHN claims to have found synapsis and a reduction of chromosomes. In Ceratiomyxa the mature spore has four nuclei. When the spore germinates there is another mitosis, and eight uninucleate swarmspores are produced from the amoeba. Swarmspores, amoebae, and plasmodia seem to have the reduced number of chromosomes, the $2x$ generation being confined to a comparatively short period in the development of the sporangium.—CHARLES J. CHAMBERLAIN.

A bacterial plant-tumor.—SMITH and TOWNSEND[27] have demonstrated that a gall occurring on the cultivated "marguerite" (*Chrysanthemum frutescens*) is caused by a bacterium, for which they propose the name *Bacterium tumefaciens*. The organism was isolated, and in some of the experiments 100 per cent. of the inoculations gave positive results, while the check plants remained free from tumors. As the authors say, "the number of vegetable galls known positively to be due to bacteria is not very great. The discovery of a new one of undoubted bacterial origin, therefore, is of considerable interest to plant pathologists, and may be of some interest to animal pathologists, especially to those interested in determining the origin of cancerous growths."—J. M. C.

Rate of growth in Jamaican forests.—SHREVE[28] has studied the rate of leaf growth in the rain-forest of the Jamaican mountains, and reaches the following conclusions: (1) that the rate of leaf growth is very slow as compared with that in tropical trees in which it has already been measured; (2) that the renewing foliage of deciduous trees does not grow more rapidly than that of evergreen broad-leaved trees; and (3) that the prevalence of conditions unfavorable to photosynthesis and transpiration would seem to offer at least a partial explanation of the slow rates of growth.—J. M. C.

Potato scab.—MORSE[29] has recently published a summary of our present knowledge of the scab of Irish potatoes and of the methods in vogue for its prevention. In addition to the well-known treatment of the uncut tubers with a solution of formaldehyde or with a solution of corrosive sublimate, an account is given of the newer treatment with formaldehyde gas generated by the heat arising from the chemical action due to pouring the formaldehyde over some

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[26] JAHN, E., Myxomycetenstudien. 6. Kernverschmelzungen und Reduktionsteilungen. Ber. Deutsch. Bot. Gesells. **25**:23–26. 1907.

[27] SMITH, ERWIN F., and TOWNSEND, C. O., A plant-tumor of bacterial origin. Science N. S. **25**:671–673. 1907.

[28] SHREVE, FORREST, Studies on rate of growth in the mountain forests of Jamaica. Johns Hopkins Univ. Circ. **1907**:no. 3. 31–37.

[29] MORSE, W. J., The prevention of potato scab. Bull. Maine Exp. Sta. **141**:81–92. 1907.

potassium permanganate. This new method gives very good results and is particularly useful when large quantities of tubers are to be treated.—E. MEAD WILCOX.

Mitotic figures.—The nature of the forces which determine the achromatic figure is discussed by HARTOG,[30] and speculations as to processes occurring in the living cell are illustrated by ingenious experiments. Finely pulverized magnetite under the influence of magnets was made to take the form of the familiar mitotic figures. Photographs of these magnetic figures showing centrosomes, centrospheres, polar radiations, mantle fibers, and central spindles are given. Tripolar and quadripolar spindles were easily produced.—CHARLES J. CHAMBERLAIN.

The xerophytic character of gymnosperms.—Miss STOPES[31] has brought together the data in reference to xerophily among the gymnosperms, and concludes "that the xerophytic characters of the Coniferales in many cases are not adaptations to xerophytic conditions in their own times, nor are they 'inherited' from the remote past as vestigial characters, but are the result of physiological limitations of the type of wood in this ancient and incompletely evolved group. In other words, their 'xerophytism' is not ecological, but phylogenetic."—J. M. C.

Embryogeny of Gnaphalium.—The occurrence of parthogenesis in Antennaria suggested to SCHILLER[32] an examination of the nearly related genus Gnaphalium. A study of *G. supinum*, *G. silvaticum*, and *G. uliginosum* showed the normal formation of four megaspores in the nucellus, a normal embryo sac with the rather extensive antipodal development characteristic of many Compositae, and the regular occurrence of double fertilization. When pollination is prevented no embryos are formed.—CHARLES J. CHAMBERLAIN.

Course of pollen tube in Sibbaldia.—According to ALBANESE,[33] the growth of the integument in *S. procumbens* continues until the micropyle is entirely closed. The pollen tube grows through the tissues of the integument and nucellus and thus reaches the embryo sac. This course of the pollen tube and also the described cases of chalazogamy are regarded not as primitive conditions but as variations from the porogamic type. Sibbaldia often shows several embryo sacs in a single nucellus.—CHARLES J. CHAMBERLAIN.

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[30] HARTOG, MARCUS, The dual force of the dividing cell. Part I. The achromatic spindle figure illustrated by magnetic chains of force. Proc. Roy. Soc. London **76**: 548–675. *pls. 9–11.* 1905.

[31] STOPES, M. C., The "xerophytic" character of the gymnosperms; is it an "ecological" adaptation? New Phytol. **6**:46–50. 1907.

[32] SCHILLER, JOSEF, Untersuchungen über die Embryogenie in der Gattung Gnaphalium. Oesterr. Bot. Zeits. **57**:137–142. *pl. 5.* 1907.

[33] ALBANESE, NICOLÒ, Ein neuer Fall von Endotropismus des Pollenschlauches und abnormer Embryosacksentwickelung bei *Sibbaldia procumbens* L. Sitzber. Kais. Akad. Wiss. Wien **113**:1–24. *pls. 1–2.* 1904.

Elementary species and hybrids of Capsella.—SHULL[34] has published an extract from his paper at the recent New York meeting of the American Association. The raising of over 20,000 "pedigreed" specimens demonstrated the presence of at least four elementary species that breed true when self-pollinated or crossed within the limits of the same elementary form. The bearing of these experimental results upon questions of plant-breeding under discussion is compactly outlined.—J. M. C.

Sporogonium of Notothylas.—LANG[35] has made a study of the sporogonium of a Notothylas, presumably *N. Breutelii*, growing in the Singapore Botanic Gardens. The embryogeny is in no way different from that of the other Anthocerotales except that more or less of the endothecium is sporogenous. LANG thinks Notothylas is a rather artificial genus which includes a number of forms derived by reduction in size of the sporogonia from one or more Anthoceros-like forms.—W. J. G. LAND.

Sporangium of Equisetum.—HAWKINS[36] has described a situation in the development of the sporangium of *Equisetum hiemale* which is hard to understand. The superficial sporangium initial divides periclinally, as usual, but the inner cell is said to be sterile, and the outer to give rise to the sporogenous tissue, which is certainly not the eusporangiate habit. With this interpretation, the sporogenous tissue is superficially exposed and forms the so-called wall-layers.—J. M. C.

Ovules of Juniperus.—KUBART[37] regards the ovulate structures in Juniperus as a single flower and not as an inflorescence, the ovules being simply transformed leaves, and the ovuliferous scale an aril. The ovulate and staminate flowers show a perfectly analogous structure. The paper contains a short discussion of the phylogeny of gymnosperms.—CHARLES J. CHAMBERLAIN.

Formative substances.—CHILD's thorough criticism of the hypotheses of "formative substances" as applied to the phenomena of development, regeneration, polarity, etc., in animals deserves attention by botanists, since like general assumptions, which involve special assumptions in an endless chain, have some currency in plant physiology.[38]—C. R. B.

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[34] SHULL, GEORGE H., Elementary species and hybrids of Bursa. Science N. S. **25**:590–591. 1907.

[35] LANG, W. H., On the sporogonium of Notothylas. Annals of Botany **21**:201–210. *pl. 21*. 1907.

[36] HAWKINS, LON A., The development of the sporangium of *Equisetum hyemale*. Ohio Nat. **7**:122–128. *pls. 9–10*. 1907.

[37] KUBART, BRUNO, Die weibliche Blüthe von *Juniperus communis* L. Sitzber. Kais. Akad. Wiss. Wien **114**:1–29. *pls. 1–2*. 1905.

[38] CHILD, C. M., Some considerations regarding so-called formative substances. Biol. Bull. **11**:165–181. 1906.

NEWS

DR. G. R. WIELAND, Yale University, is spending five months in Europe in a study of the collections of cycads.

FRANCIS DARWIN has been nominated by the council president of the British Association for its Dublin meeting next year.

SIR JOSEPH HOOKER was awarded the Linnean gold medal of the Royal Swedish Academy at the Linnean bicentenary.

DR. J. B. OVERTON, hitherto instructor, has been promoted to be assistant professor of botany in the University of Wisconsin.

F. M. ANDREWS, associate professor of botany in Indiana University, has leave of absence for a year of study in Germany.

ROB. E. FRIES has published an extended account of the life and work of Linnaeus in ENGLER'S *Botanische Jahrbücher* (41:1–54. 1907).

PROFESSOR WILLIAM TRELEASE received the degree of doctor of laws at the fiftieth anniversary of Washington University, St. Louis.

DR. MINTIN A. CHRYSLER, instructor in botany in Harvard University has been appointed professor of botany in the University of Maine.

THE GOLD MEDAL of the Linnean Society, London, has been awarded to Dr. MELCHIOR TREUB, Director of the Botanical Garden at Buitenzorg.

DR. MAXWELL T. MASTERS, editor of *The Gardeners' Chronicle*, and especially interested in the classification of conifers, died May 30, at the age of 74 years.

DR. H. HASSELBRING, University of Chicago, has been appointed assistant botanist at the Government Agricultural Experiment Station, Las Végas, Cuba.

DR. J. E. KIRKWOOD has been promoted to a professorship of botany in Syracuse University, and the subject is now recognized as a distinct department.

DR. E. W. OLIVE, instructor in botany in the University of Wisconsin, has been appointed professor of botany in the Agricultural College of South Dakota.

DR. HENRY C. COWLES, University of Chicago, left on June 20 with a party of twenty-two students, to spend six weeks in Oregon and six weeks in Alaska in ecological study.

F. E. LLOYD has been appointed special investigator in the Agricultural Experiment Station, Tucson, Arizona, to study certain problems connected with the development of the date.

DR. E. N. TRANSEAU, now at the Carnegie Station for Experimental Evolu-

tion at Cold Spring Harbor, has been appointed professor of botany in the State Normal School at Charleston, Ill.

PROFESSOR CHARLES E. BESSEY, University of Nebraska, on May 14 delivered an address on "The Place of Linné in Science" before the Botanical Club of the University of Chicago.

THE UNIVERSITY OF UPSALA, in connection with the commemoration of the bicentenary of Linnaeus, conferred honorary degrees upon W. G. FARLOW, WILLIAM CARRUTHERS, and FRANCIS DARWIN.

ERRATUM.—Three figures were unfortunately misplaced in Mr. H. D. HOUSE'S paper on "New or Noteworthy North American Convolvulaceae," in the June number. Corrections should be made as follows: For *fig. 2*, p. 411, read *fig. 4;* for *fig. 3*, p. 412, read *fig. 2;* and for *fig. 4*, p. 412, read *fig. 3*. The numbers under the three species will then refer to the proper figures.

A PLOT OF GROUND, about four acres in extent, has been set apart by The University of Chicago as a Botanic Garden. It adjoins Washington Park and fronts the Midway Plaisance, and is therefore very near to the botanical laboratory. It is to be strictly a laboratory garden, and will very much extend the facilities for experimental work. This area will be doubled as the plans develop.

THE HERBARIUM of the University of Chicago has been deposited in the Field Museum of Natural History. It contains the collection brought together by Professor COULTER during the period of his activity in taxonomy. This arrangement enables the university to offer facilities for research work in the classification of the higher plants, and incorporates with the collections of the Field Museum sets and types that will soon be made accessible for study in that admirably managed establishment.

IN THE *Bulletin of the Torrey Botanical Club* (34:167–178. 1907) an "American Code of Botanical Nomenclature" is published. It was prepared by the Nomenclature Commission of the Botanical Club of the American Association, after a study of the rules and recommendations adopted by the Vienna Congress of 1905. Exception is taken especially to the failure to recognize the principle of types, to the arbitrary selection of several hundred generic names to be exempt from all rules of nomenclature, to the treatment of homonyms, and to the requirement that after January 1, 1908, all descriptions of new species or genera must be accompanied by a Latin diagnosis. This "American Code" is in effect an announcement that the botanists signing it will not be bound by the rulings of the Vienna Congress; but how far this attitude represents American botanists remains to be seen. The BOTANICAL GAZETTE, while not in full accord with all the rulings of the Vienna Congress, is decidedly in favor of any international agreement that will bring some measure of uniformity. While the "American Code" contends for certain principles, some of which doubtless should be contended for in an international congress, the most important principle to be established is international agreement